BIOTECHNOLOGY IN THE AMERICAS:
Prospects for Developing Countries

BIOTECHNOLOGY IN THE AMERICAS: PROSPECTS FOR DEVELOPING COUNTRIES

Proceedings of a symposium held in San José, Costa Rica 3–6 May 1983

Sponsored by
Interciencia Association
Instituto Centroamericano de Investigación y Tecnología Industrial (ICAITI)/MIRCEN de Biotecnología
Consejo Nacional de Investigaciones Científicas y Tecnológicas de Costa Rica (CONICIT)

Edited by
William D. Sawyer

Interciencia Association
Washington, D.C., U.S.A.
1984

ISBN 0-918527-00-7

1776 Massachusetts Avenue, N.W.
Washington, DC 20036

CONTENTS

ACKNOWLEDGEMENT

The symposium "Biotechnology in the Americas: Prospects for Developing Countries" was sponsored by the Interciencia Association, the Instituto Centroamericano de Investigación y Tecnología Industrial (ICAITI)/MIRCEN de Biotecnología, Guatemala, and Consejo Nacional de Investigaciones Científicas y Tecnológicas de Costa Rica (CONICIT). Additional assistance was provided by the United States Agency for International Development (USAID), the Instituto Interamericano de Cooperación para la Agricultura (IICA), Costa Rica, The Andrew W. Mellon Foundation, U.S.A., and the United States National Science Foundation (NSF).

Carlos Rolz, James W. Rowe and William D. Sawyer organized the symposium. Rodrigo Zeledón and colleagues and staff from CONICIT, from IICA and from ICAITI made superb local arrangements for the symposium.

The publication of the proceedings of the symposium was made possible by a generous grant from the NSF. The efficient assistance of Alpha Med Press, Dayton, Ohio, U.S.A., under the direction of Edward Wetzel, is gratefully acknowledged. Helen Carlin and Susan Williams provided invaluable assistance in the preparation of the proceedings.

On behalf of the sponsoring organizations and the conference participants, I thank each of these groups and individuals for their fine support and assistance.

William D. Sawyer
Dayton, Ohio, U.S.A.
1984

"This material is based upon work supported by the National Science Foundation under Grant No. INT 8303825."

"Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the National Science Foundation."

LIST OF PARTICIPANTS

ARGENTINA

Juan Dellacha
Consejo de Investigaciones de la Provincia de Buenos Aires
Telephone: 84-5812

BRAZIL

Otto Jesus Crocomo
Coordenador, Centro de Biotecnologia Agricola
Fundacao de Estudios Agrarios Luiz de Queiros
Caixa Postal 9
13400 Piraciaba, SP
Telephone: (0194) 22-3491

J. R. Jardim Freire
Professor, Departamento do Solos
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Caixa Postal 776
90,000 Porto Alegre, RS
Telephone: (0512) 23-7821

Jorge Elias Kalil
Centro de Biotecnología
Rua Goncalves Dias, 580
90,000 Porto Alegre, RS
Telephone: (0512) 31-8500

Maro Ran-ir Sondahl
Department of Genetics
Instituto Agronómico de Campiñas
Caixa Postal 28
13100 Sao Paulo, SP
Telephone: (0192) 41-0511

Victor Yang
Senior Engineer
PROMON Engineering S.A.
R. Pedroso Alverenga,
220 Apto. 103
04531 Sao Paulo, SP
Telex: 11-23451 PROM BR

CANADA

Dennis G. Howell
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Telephone: (519) 824-4120, ext. 3755

CHILE

Jorge Allende
Departamento de Bioquímica
Universidad de Chile
Santiago
Telephone: 37-6320

COSTA RICA

Oscar Arias Morera
Centro de Investigaciones Agronómicos
Universidad de Costa Rica
San José

Pedro Leon Azofeifa
Centro de Biología Molecular y Celular
Universidad de Costa Rica
San José

Ludwig Muller
Centro Agronómico Tropical de Investigación Ensenanza
Turrialba
Telephone: 56-64-41

Rodrigo Zeledón
President
Consejo Nacional de Investigaciones Científicas y Tecnológicas de Costa Rica
San José

Jose Marti Solorzano
Executive Director
Consejo Nacional de Investigaciones Cientificas y Tecnologicas
Apartado 01318
San José
Telephone: 24-41-72

GUATEMALA

Carlos Rolz
Head, Applied Research Division
Instituto Centroamerican de Investigación y Tecnológia Industrial
Apartado Postal 1522, Zona 10
Guatemala City
Telephone: 310-631

MEXICO

Rodolfo Quintero R.
Director, Departamento de Tecnológia de Alimentos
Instituto de Investigaciones Biomédicas
Universidad Nacional de Mexico
Apartado 70228
0510 Mexico, D.F.
Telex: 017-74523

MEXICO (cont.)

Francisco Viliesid Alonso
Laboratorio Nacional de
Fomento Ind.
Apartado 41-537
Mexico, D.F.
Telephone: (905) 589-0199, ext. 141

Gustavo Viniegra G.
Chief of Microbiology
Division of Ciencias Biológicas
y de la Salud
Departamento de Biotecnologia
Universidad Autonoma
Metropolitana — Iztapalapa
Avenue Purisima y Michoacan,
Col. Vicentina
Iztapalapa, 13
Telephone: (905) 686-0322

SPAIN

Francisco Fernandiz
Comision Asesora de Investigacion
Cientifica y Tecnica
Ministerio de Educacion y Ciencia
Edificio Rioja, Rosario Pino 14–16
Madrid 20

TRINIDAD AND TOBAGO

John Spence
Director, Subregional Office for
the Caribbean
Economic Commission for Latin Amercia
P.O. Box 1113
Port-of-Spain
Telephone: 62-35595

UNITED STATES

Glenn B. Collins
Professor, Department of Agronomy
University of Kentucky
N-122 Agricultural Science Building — North
Lexington, Kentucky 40506
Telephone: (606) 267-2838

Emilio Q. Daddario
Past-President, AAAS
Wilkes, Artis, Hedrick & Lane
Suite 600
1666 K Street, N.W.
Washington, D.C. 20006
Telephone: (202) 467-7893

Gordon Erdman
Senior Scientist
Phillips Petroleum Company
Bartlesville, Oklahoma 74004
Telephone: (918) 661-9702

David A. Jackson
Vice President & Chief
Scientific Officer
Genex Corporation
16020 Industrial Drive
Gaithersburg, Maryland 20877
Telephone: (301) 258-0552

Cristian Orrego
Laboratory of Molecular Biology
National Institutes of Health
Building 36, Room 3D02
Bethesda, Maryland 20205
Telephone: (301) 496-6574

James W. Rowe
Executive Director, Interciencia
Association
c/o American Association for
the Advancement of Science
1776 Massachusetts Avenue, N.W.
Washington, D.C. 20036
Telephone: (202) 467-5444

William D. Sawyer
Dean, School of Medicine
Wright State University
Dayton, Ohio 45435
Telephone: (513) 873-2933

Anthony J. Sinskey
Professor of Applied Microbiology
Department of Nutrition & Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
Telephone: (617) 253-6721

Robert P. Tengerdy
Professor, Department of Microbiology
and Environmental Health
Colorado State University
Fort Collins, Colorado 80523
Telephone: (303) 491-6136

Andres Valeiras
Department of Scientific Affairs
Organization of American States
1889 F Street, N.W.
Washington, D.C. 20006
Telephone: (202) 789-3357

INTRODUCTION

William D. Sawyer

Dean, School of Medicine
Wright State University
Dayton, Ohio 45435 USA

A symposium and workshop on "Biotechnology in the Americas: Prospects for Developing Countries" was held May 3–6, 1983, in San José, Costa Rica. The symposium was sponsored by Interciencia Association, the Instituto Centroamericano de Investigación y Tecnología Industrial (ICAITI), and the Consejo Nacional de Investigaciones Científicas y Tecnológicas de Costa Rica (CONICIT) with the assistance of the U.S. Agency for International Development (USAID), the Instituto Interamericano de Cooperaction para la Agricultura (IICA), The Andrew W. Mellon Foundation, and the U.S. National Science Foundation (NSF). Carlos Rolz, James W. Rowe and William D. Sawyer were the coorganizers. Forty-two scientists from throughout the Americas participated in a series of state-of-the-art lectures, round tables and workshops. The symposium was organized (i) for consultation and analysis, and (ii) to promote the discussion of the short-, medium- and long-range prospects of biotechnology for the economic, technical and social development of countries in the hemisphere, especially in Latin America and the Carribean.

For this conference, biotechnology was defined as an applied science using biochemistry, microbiology, molecular biology, plant science, cell biology and chemical engineering to achieve industrial-scale and economically advantageous production based on microorganisms and cultivated cells. Biotechnology is a multifaceted, multi-disciplinary activity with high potential for economic development and for improvement of human and animal health. Biotechnology requires a critical mass from diverse disciplines within science and often, others such as economists and experts in marketing. Therefore, organizational and infrastructural needs were considered along with science and technology.

AN OVERVIEW OF BIOTECHNOLOGY: CURRENT AND FUTURE STATUS

Anthony J. Sinskey

Professor of Applied Microbiology,
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139 USA

This overview of biotechnology is in two parts. They are (i) the current status of biotechnology, and (ii) biotechnology and the future.

CURRENT STATUS OF BIOTECHNOLOGY

What is biotechnology? Figure 1 summarizes the biotechnology paradigm. Biotechnology as a system is concerned with converting raw materials or substrates via biological transformation in bioreactors for production and delivery of products.

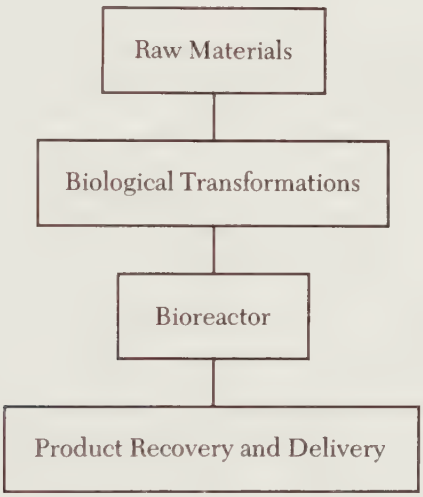

Figure 1. The biotechnology paradigm.

The next issue is the requirements for development of biotechnology. There are three key considerations. The first and the most important is intellectual capital. Secondly, focusing on relevant products and processes is required. Thirdly, manpower, managers and an infrastructure are necessary.

What is the intellectual capital that we can draw upon for the development of biotechnology as a discipline? Two scientific developments have occurred that are providing the intellectual capital for biotechnology; they are recombinant DNA (rDNA) technology with its application to biological information processing, i.e., using information in genes to make proteins, and the technology of monoclonal antibody production.

Coupled with these scientific revolutions in basic knowledge and techniques, new and novel methods are being developed for production and delivery of biologicals.

In addition, fermentation technology is becoming more of a high technology industry. Problems associated with large scale economic mammalian cell culture are being defined and strategies are being developed for overcoming them. Delivery systems for biologically active agents are becoming more sophisticated.

Figure 2 summarizes the two parallel technical pathways that are occurring simultaneously and are contributing to the development of biotechnology as a discipline. The intellectual capital is bountiful and both the scientific and engineering principles are developing rapidly. It is estimated in the field of molecular biology that there is at least a doubling of new information every three years. For some aspects of science and engineering, the doubling time may be even shorter.

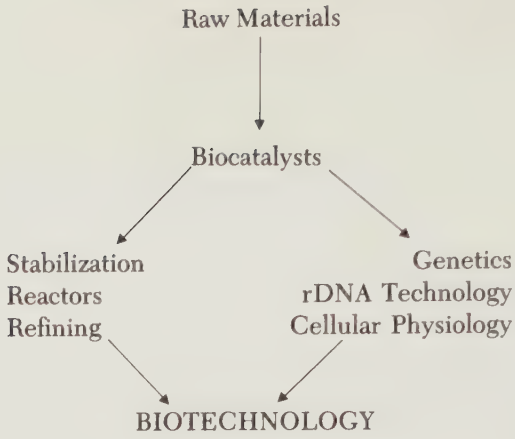

Figure 2. Parallel technical pathways in biotechnology.

Important recent developments in biotechnology include:

1. Genetic systems are being established for a wide variety of prokaryotic and eukaryotic organisms.
2. New techniques are being developed for the directed manipulation of the genetic systems of microorganisms, plants and animals, thus generating new types of organisms with specific metabolic properties.
3. New knowledge is evolving on the properties of enzymes which catalyze reactions of potential interest. It may be possible, soon, by use of recombinant DNA procedures, to generate new enzymes which catalyze unique chemical transformations. New molecules and materials can be designed and produced.
4. An aggressive high technology industry concerned with the use of modern biological methods for production of chemicals and biologicals is developing.
5. Fermentative production processes of the future will use newly designed microorganisms, cells of higher plants and cells of human origin that can utilize a variety of substrates.

Critical, however, is the necessity to focus biotechnology programs on realistic products and processes. Perhaps unfortunately, biotechnology offers solutions to almost any problem. It can lead to elegant and costly solutions to trivial problems. Some naive approaches have pursued biotransformations that were thermodynamically unfeasible. Many biotechnology solutions to problems, for example, degradation or utilization of lignin or other recalcitrant substrates, also are inherently slow with regard to rates of conversion. Such processes may not be competitive in certain countries with alternative processes.

Even with some of the unfortunate incidences in biotechnology, the first phase of development is over. What has the first phase accomplished? Practically speaking, it has primarily made protein products available as summarized in Table 1. Conversely, certain biomaterials such as polysaccharides directed to high technology applications and large scale productions are of near-term potential importance in biotechnology. Metabolic intermediates, such as organic acids or vitamins, can and are produced currently. In the future, other metabolic intermediates may find applications as unique chemicals, i.e., precursors, flavoring agents, and specialty chemicals. These will become available as both processes develop for increased productivity and the ability to manipulate the intracellular metabolic pathways increases.

Table 1. Chemicals made by biotechnology

PROTEINS (Enzymes, Antibodies, Receptors, Hormones, Antigens, Carriers, Nutrition, Functionality)

POLYSACCHARIDES (Rheology control)

METABOLIC INTERMEDIATES (Citric acid, Amino acids, Vitamins)

Note: Many final products, especially for medical applications, will not be those naturally occurring, but analogs or chemically modified versions.

The proteins being manufactured are in the form of health-care biologicals, antibodies, antigens and enzymes. Table 2 summarizes some of the protein health-care biologicals that can now be manufactured. It is important to many countries that unique antigens can be designed to combat local health problems.

Table 2. Biologicals for health care that are produced by biotechnology

Proteins: Growth factors (animal, human), Insulin, Factors XIII and IX, Tissue plasminogen activator, Interferons, Interleukins, Rennin, Relaxin, Growth hormone releasing factor, "Tumor necrosis factor"

Antigens: Hoof and Mouth Disease, Swine and Cattle Scours, Hepatitus (B, A, non-A-non-B), Influenza, Pertussis, Cholera, Dysentery, Malaria, Herpes, Gonorrhea, Animal diarrheas

Returning to the biotechnology paradigm, let us examine some of the current issues, and how they are formulated and analyzed.

RAW MATERIALS

Technical opportunities abound in the area of raw material utilization, and many are being explored for applications in the energy, food and chemical industries (Table 3). Many of these opportunities are very relevant to coun-

Table 3. Utilization of new materials

Raw Materials	Relevance to Biotechnology
Efficient utilization	Xylose, Cellulose
Ingredient substitution	Starches, Proteins, Lipids
Availability	Gums
Functionality	SCP, Proteins

tries rich in renewable resources. For example, organisms can now be genetically engineered to ferment substrates which have not been ordinarily utilized. One such example is the fermentation of xylose into ethanol by *Saccharomyces cerevisiae*. Other renewable resources such as cellulose can be more efficiently utilized by developing a variety of organisms with improved cellulytic capabilities. New molecules could be designed to substitute as ingredients with unique functionality in a variety of food and health care systems.

There is an opportunity to make some raw materials more readily available via applications of modern biology to plants and other biological systems. Major efforts are oriented toward this activity in the U.S.A. Genetic solutions to drought resistance and salt tolerance of plants are being extensively researched.

BIOTRANSFORMATIONS

The heart of any biotechnology process is the biotransformation, i.e., the catalysis step (Table 4). One can

Table 4. Biological transformations

Catalysts	What product?
Enzymes	What activity?
Whole cells	What cofactors and substrates?
• Plants	What strains?
• Animal	Strain improvement techniques?
• Microbial	• Classical genetic procedures
	• rDNA
	• Microbial physiology

choose from a variety of available catalysts. These include isolated enzymes and whole plant, animal or bacterial systems. The choice of the biocatalyst depends upon the product in question, the substrate to be utilized, the cofactor requirements, and whether or not post-translational modifications of products are required. When these questions are answered, specific catalysts become obvious and the strategies for their improvement clearer (Figure 3). In Figure 3, we see that enzymes are being used for simple chemical conversion usually where chemical

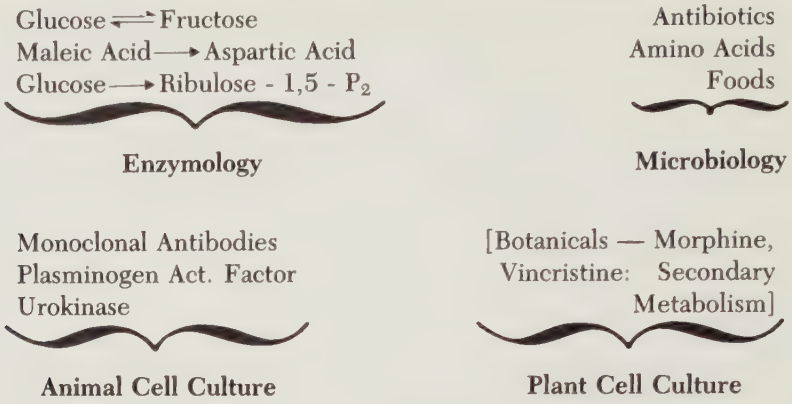

Figure 3. Catalyst strategy.

stereospecificity is desired. Microbes on the other hand are used for the production of more complicated molecules (antibiotics, proteins and vitamins), expecially where multiple synthetic steps are required. Mammalian cell cultures are important sources of monoclonal antibodies and proteins requiring post-translational modification, while plant cell culture currently provides limited opportunities to develop processes for secondary metabolites including oils and triglycerides. For any of these biotransformation processes, barriers to process development and scale-up have to be overcome. For reference, some of the more important technical barriers in mammalian cell culture are summarized in Table 5:

Table 5. Barriers to scaling-up mammalian cell culture techniques

BARRIERS TO SCALE-UP THAT STILL NEED TO BE ADDRESSED:
Oxygen-transfer limitations
Accumulation of toxic waste products
Lack of regulatory hardware and software for process control
High cost of serum
BARRIERS TO SCALE-UP THAT HAVE BEEN ADDRESSED TO SOME EXTENT:
Low surface-to-volume ratios of systems that cultivate anchorage-dependent cells
Shear sensitivity of mammalian cells

BIOREACTORS

Chemical engineering principles relevant to bioreactors have to be further defined and developed to improve on the most important parameters of any biotechnology process — yield, productivity or both. New instrumentation and process control strategies will be required to achieve these goals. More engineers are needed who can communicate effectively in the rapidly changing language of biological sciences. Lacking to date are engineers capable of evaluating the technical opportunities in the biochemical process industry.

PRODUCT RECOVERY AND DELIVERY

Related to bioreactor developments, new and more effective technologies are required for isolation of organic chemicals from fermentation broths. Novel and low cost procedures applicable to large-scale process are also required for isolation and recovery of protein products. Since one of the main products of this first phase of biotechnology has been health-care proteins, new procedures are needed to purify and deliver these macromolecules.

EFFECTS OF BIOTECHNOLOGY ON DEVELOPMENT IN THE AMERICAS

A variety of possible effects on a given country's advances will result from the recent developments in biotechnology. A most important fact is that there are unique markets and targets available for solution by biotechnological principles in specific countries in the Americas. These include:

1. The fact that many countries are rich in natural resources, some of which are renewable. Examples include many kinds of agricultural biomass. Processes may be developed for them that are not capital intensive.
2. Unique human and animal health-care problems exist in defined locations in the world. Foot and mouth disease and specific parasitic problems can be found in different parts of the world.
3. Solutions or improvements in food supply and efficiency of land use can now be rationally developed.
4. Renewable resource conversion to liquid fuels may reduce energy bills for a developing country.

Not all effects may be positive. For example:

1. For many countries there is a manpower shortage, expecially of scientists and engineers who can address relevant problems. How to develop and maintain manpower becomes a recurring problem.
2. Balance of payments may be negatively affected if a country has to pay new food, health and energy bills based upon developments in biotechnology.
3. There is an information as well as technical gap in transfer of biotechnology from western countries to other parts of the world. Currently, the time constant in the U.S.A. for basic research to be transferred to the industrial setting for development is extremely short.
4. Many of the problems in biotechnology are high risk science. Success is not guaranteed.
5. University and industrial linkages have to be strengthened.
6. Financing of biotechnology research and development is expensive and requires not only traditional methods but newer and more creative financing including use of tax shelters, research and development partnerships, and others. These are not readily available in many countries.

Effective and dedicated managers and planners will be required to minimize the negative effects of many of the recent developments in biotechnology on a given country's development.

TRANSFER OF BIOTECHNOLOGY

The barriers to biotechnology developments are listed in Table 6. A variety of techniques will be required to

Table 6. Barriers to biotechnology development

Human resources
Focused problems
Research and development facilities
Financial
Relevant scientific data base
Legal and regulatory

overcome these barriers in order that biotechnology principles can become more relevant to operations in developing countries, including training programs, joint research programs, and other standard projects. Programs such as the Advance Technology Alert System (ATAS) of the United Nations are likely to become more important.

A high priority is to establish an effective infrastructure in countries that can absorb, evaluate and assign priorities to the national biotechnology development needs. What is needed most is qualified experts committed to solving biotechnology problems relevant to local needs. Current scientific and economic rewards associated with many of the challenging biotechnology problems in certain countries are not competitive with other opportunities in biotechnology for the top scientists. The brain drain that some countries are facing in this area has reached a critical stage and may in fact now be hindering developments in biotechnology. This intellectual gap and its resulting economic constraints represent the greatest barrier to transfer of biotechnology to developing countries.

It is essential to have a critical mass of well trained scientists working in well equipped laboratories in order to remain competitive. This obviously requires a long term financial commitment. In addition, there is need for technical personnel well trained in the fundamentals of science and engineering who are capable of integrating and evaluating important breakthroughs in a variety of disciplines.

A critical mass of scientists has to be trained to develop the second and third phases of biotechnology in a given country; the ability to solve internal science and engineering problems will be extremely unlikely unless a critical mass of human intellectual resource is developed and maintained. Without a critical mass of scientists and engineers, the impact of biotechnology in such countries is likely to be minimal.

BIOTECHNOLOGY AND THE FUTURE

The second phase of biotechnology is now occurring (Figure 4). It is, in part, concerned with the development

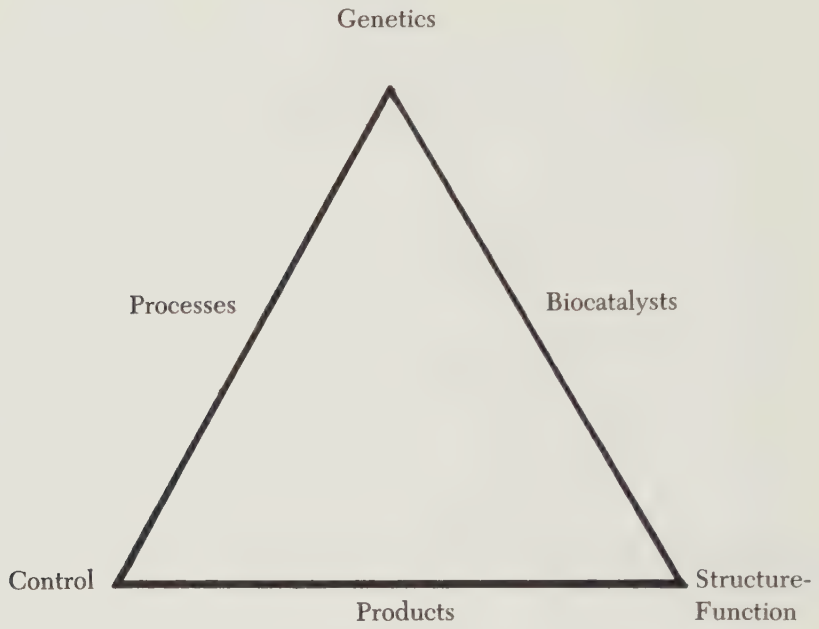

Figure 4. Modern genetics and industrial organisms.

of modern genetic systems appropriate to industrial organisms. This phase of development is also likely to be significant to a variety of national needs. Many unique organisms are used in both food and fermentation processes in many parts of the world. Much modern biological knowledge (both genetics and physiology) is lacking for these organisms. Efforts on the application of genetics to improve yields, productivity and utility of biologically derived products from such organisms is likely to be an important area for further development. Current research efforts in our laboratories with *Corynebacterium glutamicum* and *Lactobacillus*, organisms which have been traditionally used in many processes but have not been subjected to rigorous modern biology approaches, are resulting in new genetic systems to improve the utilization of these organisms. In addition, rDNA systems that are environmentally sound will have to be developed for such organisms. There is a real need for genetic systems for food-grade organisms that pose no health hazard.

In the third phase, efforts will be on how to control and establish the structure-function relationships of proteins and other macromolecules such as polysaccharides. As summarized in Figure 4, significant scientific developments have occured in relating structure-property relationships with proteins or enzymes. One is able to foresee wide applications based on these basic principles. An enzyme's catalytic site may be altered. Thermal as well as solution properties of the macromolecules may be manipulated to make the macromolecule properties more useful for a variety of applications.

The fourth phase will be concerned with the development of procedures where the fundamental driving force for this field, i.e., biological information processing, can be truly controlled. Eventually, successful biological control strategies will allow scientists to turn on or off the molecular switches for controlling and regulating specific genes for development. Some specific examples relevant to Figure 4 include developmental biology, plant science and the biochemical processing industry. A variety of stage processes will result from the formulation of basic principles for control of biological information processing.

ECONOMIC SECTORS

Future developments in biotechnology will have major impact in a variety of sectors including health-care, agriculture, animal nutrition and health, plant tissue culture, food, marine and aquaculture, energy, corrosion control, metals, specialty chemicals, military, delivery systems, and biomaterials.

For most countries, developments in food, plants, health and energy are likely to be the most important. Important developments likely to occur in each of these areas are briefly summarized in Table 7.

Table 7. Likely applications of biotechnology

FOOD

U.S. Market: Convenience
Low-calorie
Safety ("Natural foods")

Food Quality and Safety — Analysis

Flavor Enhancement

Cheese, Cocoa, Coffee, Tea, Yogurt, Beer, Wine, Soya, Fish sauce: Improved rate of flavor production, High-impact flavor additives

Agents for Texture, Rheology Control
Carragheenan

Substitution of Raw Materials (Soya protein for milk or meat)

PLANT TISSUE CULTURE (Crop, Location, Specific Problems)

Disease (Virus) Elimination:
Meristem or Shoot-Tip Culture
Clonal Propagation
Breeding Programs (Sterile lines, Vegetatively propagated species, Slow-growing plants, Elite germ plasm)
Mutants (Selective agents, or naturally through culture)
Amino acid overproduction, Disease resistance, Herbicide salt, heat, cold, metal tolerance
Gene Transfer
Related but sexually incompatible species, Widely different species (Protoplasts)
Chemical Production
Morphine, Vincristine/Vinblastine, Flavors and Odors

ENERGY

Oil Production

Polysaccharides for viscosity control, Oil-water interfacial tension lowering, Chemically enhanced oil recovery

Heavy Crude and Coal Slurry Pipeline Transport and Burning
Cleaning and Environmental Maintenance
Biomass Production: Ethanol (Distributed technology; Methane (Municipalities and large farms); Synthesis Gas
Thermal Ocean Gradient (Fouling, Corrosion control)

FOOD: SPECIFIC PROCESS PROBLEMS

Phenylalanine for Aspartame
Cocoa Butter
Cheddar Cheese Flavor
Rennet Substitutes

High-Temperature Glucose Isomerase
Flavor Release from Chewing Gum
Vitamins (C, B_{12}, E, — Carotene)
Acidulants

ANIMAL NUTRITION/HEALTH

Amino Acids (Lysine, Tryptophane, Methionine)
Processing of Foods to Improve Utilization (Silage)
Trace Elements
Animal Food Antibiotics
Growth Hormones, Growth Hormone/Development Releasing Factors — Delivery Systems
Animal Vaccines — (Newcastle Disease: Delivery systems)
Vitamins

SUMMARY

An effective infrastructure is needed to define markets and put together an outstanding technical team for solution of relevant problems. The critical issue appears to be the lack of technically trained persons in this rapidly developing field. The field of biotechnology is real. A new discipline analogous to the scientific and engineering developments in chemistry and chemical engineering will be born. This will happen when many bureaucratic barriers are overcome. The current problem is how does one manage this most powerful knowledge base with all its possible ramifications.

ACKNOWLEDGEMENTS

The author is grateful for constructive criticism by his colleagues at MIT and from the Laboratory of Applied Microbiology (especially C. Batt, W.S. Shanabruch, S. Picataggio). Studies on mammalian cell culture were supported in part by a NCI Training Grant 5-T32-CA09258-05.

BIOTECHNOLOGY: TRADITION, TECHNOLOGY AND TARGETS

David A. Jackson

Vice President and Chief Scientific Officer
Genex Corporation
Rockville, Maryland 20877 U.S.A.

Biotechnology is not new. Man has been utilizing biologically based technologies since the dawn of civilization, if not before. Wine, beer, cheese and bread are all examples of food stuffs in which a processing step mediated by a microorganism is essential. Within the past century, there has been a rapidly accelerating use of biologically or biochemically based processes in a wide variety of applications. Major new industries, such as the production of amino acids or antibiotics by fermentation or the production of high fructose corn syrup by immobilized cell or enzyme processes, have resulted. Each of these three industries has a world market well in excess of US $1 billion per year.

Within the past decade, several fundamentally new technologies have developed which have enormous potential for impacting traditional biotechnology. The first of these is genetic engineering or, more properly speaking, recombinant DNA methodology. The second of these is biochemical engineering, particularly as it relates to the use of processes based on immobilized cells or enzymes. The challenge in biotechnology today is to form a productive combination of these new technologies with the traditional biotechnology-based industries which include agriculture, food processing, human and animal health, and portions of the chemical industry. In suggesting how this challenge can be met, I will focus on four factors:

1. the importance of developing a critical mass of skilled individuals,
2. the necessity for coordinating and integrating a variety of technical and business skills,
3. the importance of defining and focussing on specific objectives, and
4. the necessity for choosing objectives with great care.

In this analysis, I will refer occasionally to how these factors have been addressed by the new biotechnology companies which have been formed in the United States and elsewhere during the past six years. It is likely that any significant initiative in biotechnology in the Central and South American countries and the Caribbean countries, whether in the form of a commercial enterprise, a government research institute or an academic consortium, would encounter many of the same kinds of problems such companies have had to face during their transition from corporate infancy to corporate adolescence.

It is useful to begin a discussion of the opportunities for biotechnology by examining the roots of this technology. What were the disciplines which contributed most heavily to the dramatic advances of the past decade (Figure 1)? If we focus on genetic engineering, the key disciplines were the chemistry and biochemistry of nucleic

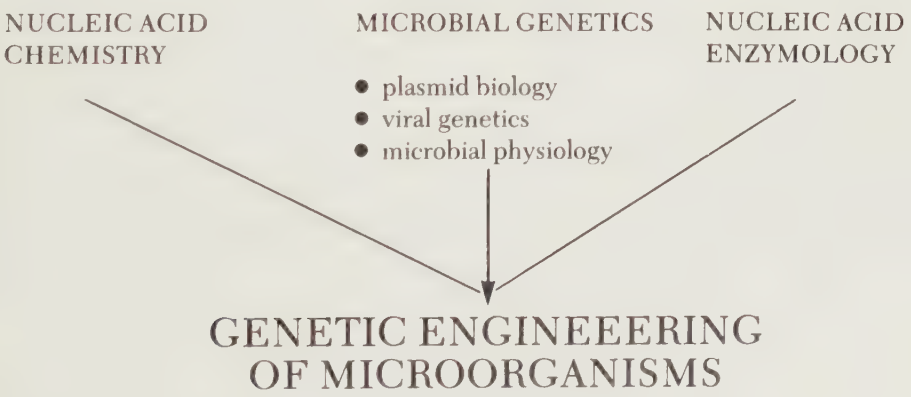

Figure 1. Development of biotechnology.

acids, especially DNA; the biochemistry of a large number of enzymes which act on nucleic acids; and the development of microbial genetics, which led to a series of highly sophisticated and easily utilized procedures for

genetic exchange in microorganisms. Each of these disciplines developed for many years largely independent of the other two. Each plays an essential role in recombinant DNA technology. Their coming together in the early 1970's resulted in the invention of this new and powerful technology.

But genetic engineering, which is a necessary component of many processes in biotechnology, is not sufficient by itself to permit application of biotechnology (Figure 2). For this, one needs at a minimum the kinds of

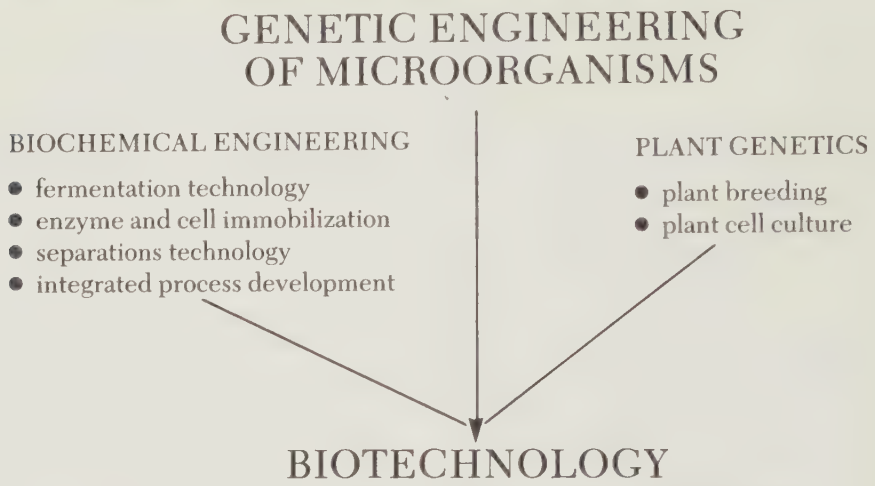

Figure 2. Requirements for application of genetic engineering to biotechnology.

biochemical engineering and fermentation technology skills that are used in producing, recovering and purifying products on a large scale. To extend the applications to the agricultural area, plant geneticists and physiologists are also essential.

In general it is these disciplines, from which the biotechnology industry grew, which are still needed to provide the proper mix of technological competencies required to achieve a functioning critical mass. What then are the skills which are currently required to achieve a functioning critical mass? The answer depends on what applications technology will use. However, for a wide variety of products produced by fermentation or by immobilized cells or enzymes, the minimum set of technical competencies is shown in Table 1. In many cases a single indi-

Table 1. Disciplinary components of critical mass required for genetic engineering of microorganisms and applications in the fermentation industry

CHEMISTRY
- Oligonucleotide synthesis
- General analytical chemistry

BIOCHEMISTRY
- Nucleic acid enzymology
- Nucleic acid biochemistry
- Protein isolation and characterization

MICROBIOLOGY
- Microbial genetics (bacteria, phage, plasmids)
- Microbial physiology

FERMENTATION TECHNOLOGY
- Applied microbial physiology
- Product recovery
- Integrated process development

vidual will have competence in more than one of these areas. Nonetheless, there is quite a wide diversity of skills that is required, and the absence of any one is likely to prove a bottleneck for the entire operation. If the technical group is to be the nucleus of a commercial enterprise, individuals with the requisite business skills in marketing, commercial development, finance, etc. must also be added to achieve a critical mass.

However, as in any enterprise consisting of more than one person, it is not enough simply to have people representing the required diversity of skills on hand. Active efforts must be made to ensure that the activities of these people are effectively coordinated and integrated. This is not as easy in practice as it may sound. The difficulties tending to inhibit effective integration of efforts may be especially prevalent in biotechnology for several reasons. First, many of the people coming into the commercial aspects of this field have either not worked in businesses before or, if they have, have not held senior managerial positions. Thus, the new biotechnology industry tends to be managed at present by people who are relatively inexperienced commercially. Secondly, most of the technical staff in the areas of molecular biology, biochemistry, microbiology, etc. come to these new enterprises directly from universities or research institutes. In the university setting, there is relatively little importance placed on being able to work as a member of a large, multi-disciplinary team which must achieve specified objectives in a specified length of time. Thus, many of the people with the technical expertise required by the biotechnology industry are not used to working in the highly integrated organizational setting required for success in this (and other) industries. Thirdly, because such a diversity of talents are required, individuals with very different backgrounds and different professional cultures must communicate effectively with one another. At first, this is often difficult.

These difficulties are all ones that can be resolved, particularly if they are anticipated and solutions are developed in advance. However, they do place a very high premium on the ability to coordinate and integrate diverse sets of skills.

I would now like to consider applications made possible by the new developments in biotechnology. Rather than describe a list of specific products for which economic production will be made possible or at least more efficient by biotechnology, I would like to focus on generic advances which have been made possible. For example, genetic engineering has made possible a whole set of what might be termed "product improvements" (Table 2)

Table 2. Improvements from genetic engineering

MICROORGANISMS — PRODUCT IMPROVEMENTS
Production of novel products
Proteins (enzymes, hormones, vaccines)
Primary metabolites
Secondary metabolites

MICROORGANISMS — PROCESS IMPROVEMENTS
Faster rate of production
Higher yield from feedstock
Higher titer of product
Utilization of novel feedstocks

by virtue of its ability to introduce the gene for any protein from any organism into a microorganism. The microorganism can thus be transformed into a "mini-factory" for the cheap and efficient production of the protein coded for by the gene. This is an example of a generic application which can be applied to many products for many industries: pharmacologically active proteins such as interferons or lymphokines for the pharmaceutical industry; enzymes such as rennin or pectinases for the food processing industry; growth hormones and vaccines for human and animal health, and enzymes for the production of specialty chemicals such as amino acids. Other and equally important generic applications of biotechnologyy are what might be termed "process improvements" (Table 2). Both conventional genetic techniques and recombinant DNA methodology can be utilized to construct microorganisms which produce traditional products, such as amino acids or vitamins, at higher efficiencies. Novel biochemical engineering approaches also make it possible to produce a range of traditional products at high efficiency and, therefore, lower costs. Improvements in the rate of a production of a product, the yield of product from feedstock or in product recovery, the titer of product in process streams, or the abiltiy to utilize alternative and cheaper feedstocks are all aspects of process improvements made possible by advances in biotechnology. Examples of how these and other generic improvements made possible by biotechnology might be applied in a

number of major sectors of the economy are listed in Table 3. Within each of the categories listed, there are a variety of specific products.

Table 3. Industrial applications of biotechnology

PHARMACEUTICAL INDUSTRY
- Novel products
- More efficient production of conventional products
- New diagnostic tools

CHEMICAL INDUSTRY
- Alternative production processes
- Alternative feedstocks

FOOD PROCESSING
- Cheaper additives for better nutrition
- Enzymatic processing

AGRICULTURE
- Crop protection
- Crop improvement
- Improved animal nutrition
- Animal protection

ENERGY
- Use of renewable resources
- Tertiary oil recovery
- Methane from biological waste

Which product or set of products makes most sense to develop will be influenced by a whole set of factors, ranging from technical to economic to local needs to local resource base. Choosing the appropriate objective or set of objectives to work towards is in many ways the most important decision of all those that must be made. Questions such as the following need to be carefully considered.

1. What are the markets for the products being considered?
2. What are the competing products and technologies serving those markets or capable of doing so in the future?
3. Is there a local technological edge, or can one be developed rapidly and economically?
4. Is there a local resource edge?
5. Can a family of similar products utilizing the same or related research and development efforts be identified?
6. Can portions of the required technology be acquired elsewhere under favorable terms?
7. What are the capital requirements?
8. Where will the capital be obtained; private sector, governments?
9. What is the anticipated development time?
10. How will the products be distributed and marketed?
11. What will be the impact of lowered selling price on market size if production costs can be lowered?

One of the most important outcomes of such an analysis should be a decision as to where to focus research and development efforts. A focussed effort is extremely important. In general, it allows the most rapid development of a high level of R & D capability, it requires a smaller size critical mass, and it allows substantial economies if a family of products can be developed from a single R & D program. For example, Genentech has established a position of leadership among the new biotechnology companies concentrating on pharmaceuticals. It has done this by concentrating the majority of its R & D efforts on what is basically a single technology, that of cloning and

expressing the genes for eukaryotic proteins at high efficiency. Another example of a focused R & D program is shown in Figure 3. This is the one adopted by Genex Corporation. It focuses on the central importance of en-

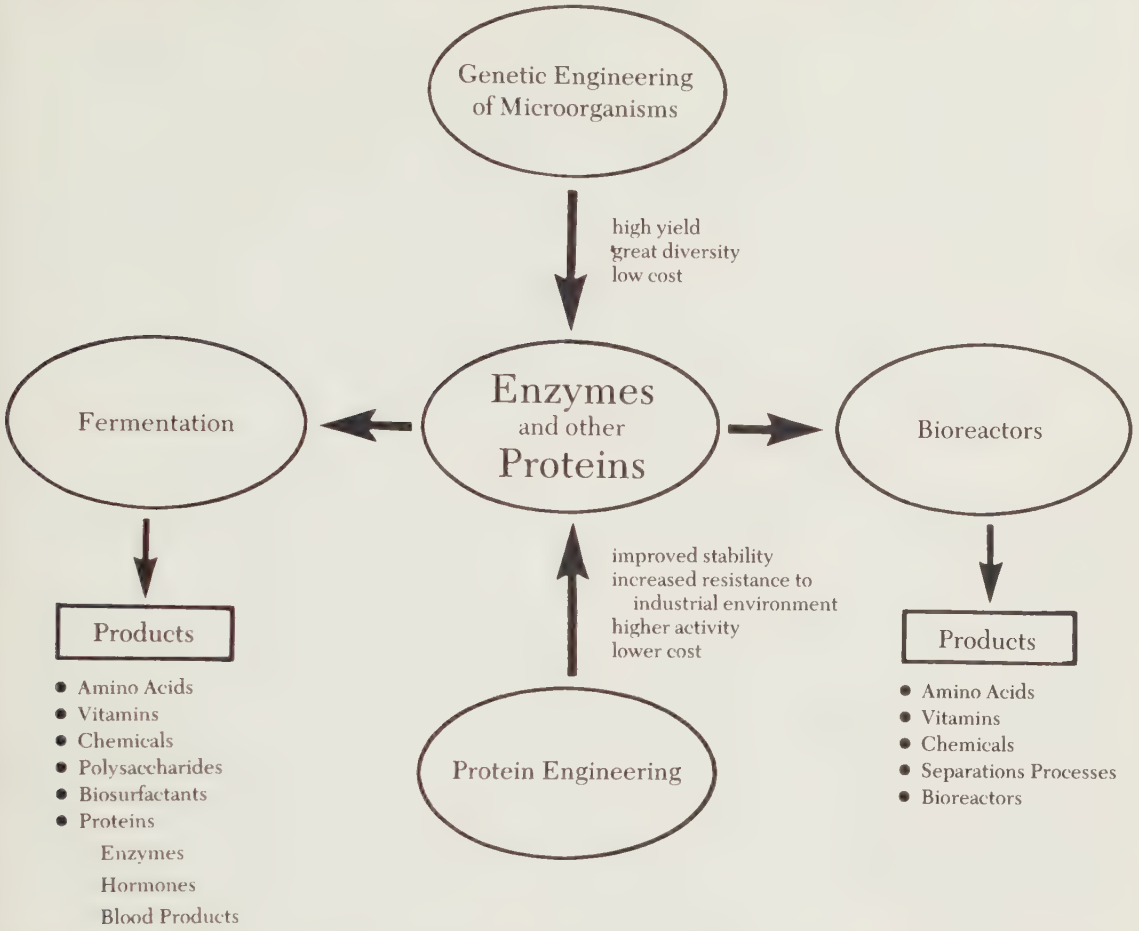

Figure 3. Enzymes in biotechnology

zymes in biotechnology, especially for industrial applications. Enzymes have existing uses in both traditional and developing technologies. Since one of the major inhibitions to the use of enzymes has been their high cost, markets for enzymes should expand as their cost is lowered, and entirely new product lines should become economically viable. In addition, several new technologies have great potential for expanding the diversity of enzymes which can be produced cheaply and for enhancing their value.

In summary, applying biotechnology successfully involves a number of strategic considerations (Table 4). The

Table 4. Strategic Considerations

Focus on specific, limited objectives or technologies
Chose objectives with great care, e.g.,
- expanding markets
- lack of competition
- commanding technological lead
- solutions possible with limited new technology
- R & D for different objectives overlaps

Define and develop critical mass from relevant disciplines

Integrate and utilize technology developed elsewhere

most important of these are choosing specific, limited objectives; focusing on these objectives; defining and developing the critical mass required to achieve them; and integrating the various skills and technology needed to accomplish the goals in a timely and economic fashion.

BIOTECHNOLOGY: AN OPPORTUNITY FOR THE AMERICAS

J. Gordon Erdman

Senior Scientist in Research and Development
Phillips Petroleum Company
Bartlesville, Oklahoma 74004 U.S.A.

Ann C. King

Technical Writer
Phillips Petroleum Company
Bartlesville, Oklahoma 74004 U.S.A.

Biotechnology is more than a subdiscipline of biology. The field provides new ways to solve problems, as well as a whole new way to produce desired products or to enhance existing production. An analogy used by Dr. Philip Abelson, the editor of SCIENCE, explains just what biotechnology is.

He says that, in the laboratory a microorganism can grow in a culture. The microorganism will eat the substrate provided and from it will make whatever chemical components it needs to sustain life. If a specific amino acid is supplied in the medium, the microorganism will stop synthesizing that amino acid and use that supplied in the medium. If, however, the amino acid supply is deleted, the microorganism will begin synthesis again. This ability invokes great respect for microorganisms as chemical engineers. Viruses have developed this selective synthesis ability one step further. Some viruses can maneuver a microorganism into making whatever is necessary to sustain and reproduce the virus itself. Humans have now become almost as smart as the viruses. We are learning, through biotechnological skills, how to utilize microorganisms to supply ourselves with biomedical products, hormones, food and feed supplements, new monoclonal antibody testing methods, vaccines, and many more. They may even be induced to make "spare parts" for the human body.

Biotechnology, therefore, is a going concern. The who, what, when, where, why, and how of the industry are as follows:

1. WHO? You, the Americas and the Caribbean countries
2. WHAT? Biotechnology
3. WHEN? Now
4. WHERE? Locally, in your own countries and companies
5. WHY? Non-capital intensive research and industry development
6. HOW? The subject of this article.

Each of the Central and South American countries and the Caribbean countries has excellent scientists and engineers. These scientists and engineers are uniquely well qualified to recognize application opportunities in current biotechnology research. These scientists know which applications might benefit their countries — local applications which may well be overlooked in the United States, Canada, Japan and Western Europe, where the needs of these countries may take priority.

It is to everyone's advantage, however, to learn and benefit from the organizational and investment successes and failures in these countries. One well publicized economic mistake was the attempt by E. F. Hutton to use a biotechnology laboratory to generate a tax shelter for investors without establishing clear scientific objectives. The company, DNA Sciences, folded and has been revived as California Biotechnology, Inc.

Some other companies in the United States have gone bankrupt or are regrouping their efforts in an attempt to stay in business. However, the large majority of the some 200 biotechnology companies founded within the last five years, are viable concerns.

The most significant change the industry has undergone in the United states is a transition in the type of personnel in outside contact positions from research scientists to business talent. From the point of view of the Americas, this bears out the argument for the need of both scientists and business people in this industry. As E. F. Hutton found out, business people alone cannot succeed in science and now the rest of the industry is finding that scientists alone cannot succeed in business.

There are two strategies for success in a biotechnological endeavor. Remember that the ultimate goal is to produce a marketable product in your home country with potential for export.

The first strategy toward this end is finding and developing a niche market. This strategy has worked very well throughout the world for small companies. Direct competition with DuPont, Eli Lilly, Monsanto, ICI, etc. would be difficult. The large organizations will usually win out in a broad front approach. However, small companies can seek a specialty medical or chemical market of perhaps only $1 million a year, one too small for big companies to develop. With careful selection, a small company can develop two or three of these "niche" markets, be assured of their sales and be free from competition.

In the Latin Americas and the Caribbean, this approach would work well because local scientists are able to identify and concentrate effort on research of local importance which is being neglected by the large organizations.

The second strategy for business success in biotechnology requires a very high level of creative insight. It is a "leap frog" technique. The method is as follows: (i) single out research already in progress for which there is a local application; (ii) assess whether and when the research is likely to be successful and ensure that the results will, in one way or another, be available; and (iii) then initiate a research program to devise an application of the forthcoming result. When the results of the original research are achieved, the application will be ready for immediate implementation.

For example, growth hormones for chickens and cattle, among others, are being developed now. However, the key to the economic success of the products will be an effective method of mass administration to these species. This is work that could be undertaken now pending success by the different laboratories with the development of the growth hormones.

Irrespective of the strategy, there is a "critical mass" of technical information, trained personnel and equipment needed for success. And there are a number of ways to obtain this "critical mass."

1. Buy or license the basic technology. Concentrate on adapting and applying it to your specific local need or needs. There are many biotechnology companies around the globe which have sprung out of, and maintain close ties with, strong academic research groups. The companies are in urgent need of income and, in most instances, are willing to sell or license their technology on reasonable terms. Larger companies are also often willing to license newly developed technology. At present, biotechnology seems to be long on technology and short on applications.

2. Buy into a small biotechnology company. Obtain, thereby, access to a desired technology and at the same time gain their strength in the desired area of expertise.

3. Establish a joint venture company with an outstanding research organization or buy into an existing joint venture. This is one approach taken by Phillips Petroleum Company which is proving highly successful. In Phillips' opinion, the non-profit Salk Institute for Biological Studies in La Jolla, California, possesses one of the greatest concentrations of experience and staff expertise in the United States, if not the world. Phillips has experience in fermentation technology and is strong on engineering. Hence, Phillips and Salk established a joint venture, named The Salk Institute of Biotechnology/Industrial Associates (SIBIA). This organization, for profit, carries on leading-edge research of mutual interest to the Salk Institute and its industrial associates. In such organizations as SIBIA, an industrial associate may also carry on approved programs for its own benefit. A country or group within a country might apply for, be accepted and pay a fee to become a member of an existing joint venture or establish their own with a company, organization or university. In such an arrangement, the results of studies carried out for a participant can be protected by patent once the complex technical problems have been solved. Development and application could then be carried out in your home country.

4. Establish a pan Central-South America, Caribbean biotechnology research center to carry out leading-edge research. Individual countries, groups of countries or enterprises within countries could contract developmental research for their benefit prior to moving the resulting project to their own facilities for application and scale-up.

   The United Nations Industrial Development Organization (UNIDO) has begun to evaluate potential sites for such a center on a worldwide basis. Dubbed the "Center for Genetic Engineering & Biotechnology" (ICGEB), the center is expected to cost in the neighborhood of 38.5 million dollars. Countries vying for the center include Belgium, Cuba, India, Italy, Pakistan, and Thailand. The final decision will be made in July 1983 in Madrid. This type of center would have several good aspects as well as several points of

contention. The geographic coverage may be too large and the diversity of languages and objectives too great.

5. Establish biotechnology research centers in separate countries.

The last two approaches to acquiring the necessary technology to venture into the biotechnology industry are the most satisfying, but, require major commitments of money and scientific manpower. Also to plan and establish such a center and to get it into operation requires time. Accordingly, it might be advisable to consider these centers in conjunction with one of the earlier options on an interim basis.

As an academic, scientific pursuit, and as a basis for industries, biotechnology is well suited to exploitation in the Central and South American and Caribbean countries. While the highest level of scientific knowledge and, in its commercialization, business acumen are needed, large amounts of capital are not needed. Nor are great quantities of natural resources required. Laboratory buildings and equipment requirements are relatively modest. Some means of containment, usually P-1, is desirable and sufficient. Facilities for handling low levels of radioactivity are needed. An exception is work with pathogens where more stringent containment is necessary. Fermentors and separation equipment represent the core equipment.

In terms of U.S. dollars, a small manufacturing plant and supporting laboratory might be assembled for 10 million dollars or less depending on locally available materials and fabrication and construction skills. Feedstocks, such as methanol, ethanol or simple sugars, can be derived from locally available fossil fuels or renewable agricultural or forestry biomass. Figure 1 shows inter-related fossil and biomass feedstocks for food and feed ingredients, pharmaceuticals and specialty chemicals.

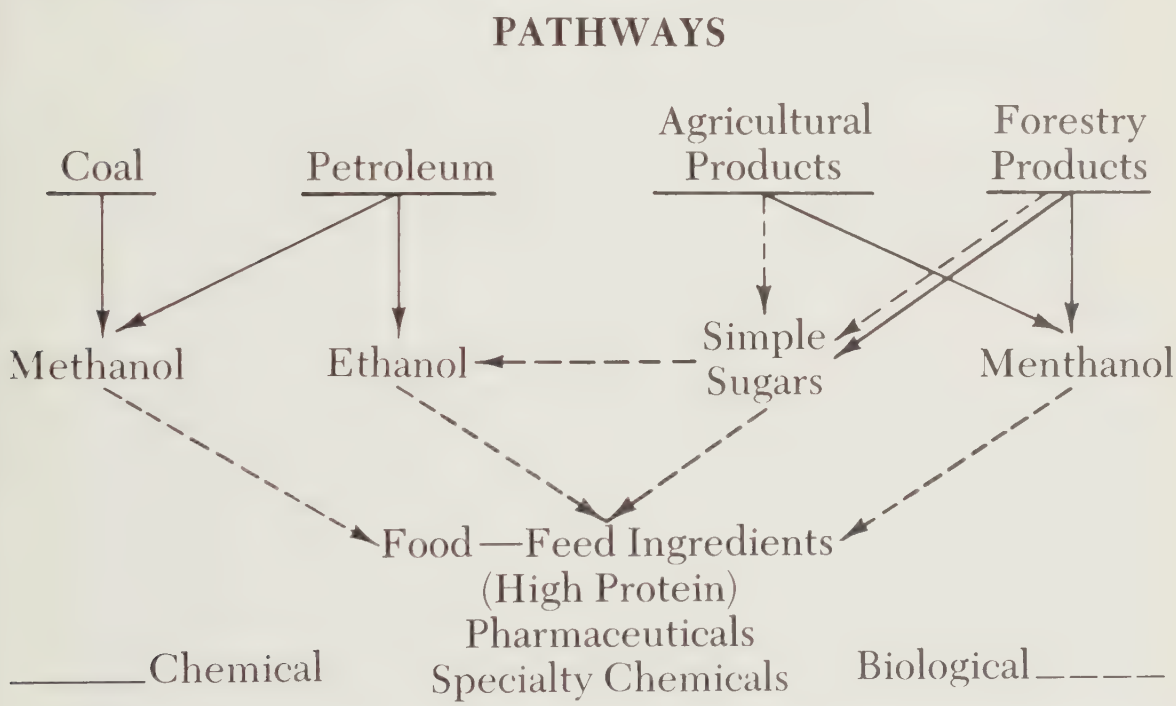

Figure 1. Chemical and biological pathways from fossil and biomass feedstocks to food and feed ingredients, pharmaceuticals and specialty chemicals.

The entire research process in biotechnology is considerably lower in cost than such highly capital intensive areas as nuclear fusion. In nuclear fusion research, as in biotechnology, the highest level of scientific expertise is required. But unlike biotechnology, testing of theory and development in nuclear fusion is very expensive. To test, for example, the pinch theory in nuclear fusion, a machine costing tens of millions of dollars is required. A facility with the chance of achieving break-even, that is minimal self-sustained fusion, probably will cost in the hundreds of millions of dollars. In addition, the first prototype power facility, and if successful, the plants to follow, will cost billions of dollars per unit. Even the more mundane industrial enterprises are often capital intensive. In another example, runs to test gasification of coal may involve 25,000 tons of coal and even to manufacture polyolefins requires a capital intensive refining and chemical complex to provide the ethylene and propylene feedstocks.

In contrast to these conventional industries and their products, the products based on biotechnology are of small volume and high value per unit weight as shown in Table 1. Two exceptions may come in beverage and fuel

Table 1. Potential markets for biotechnology products

MARKETS	PRODUCTS	$ POTENTIAL	METHODS
Agriculture	Superior New Varieties higher yield better quality Uniform Stocks clones (tissue culture) Virus Free Seed Improved Survival Characteristics virus, fungus, pest resistance herbicide resistance (allelopathy) stress tolerance	>$30 billion	Tissue Culture Cell Fusion rDNA
Specialty Chemicals	Ethanol Fructose Sorbitol Ethylene Glycol Propylene Glycol Ethylene Oxide Propylene Oxide Citric Acid Fumaric Acid Gluconic Acid Lactic Acid Propionic Acid Sorbic Acid Tartaric Acid Pheromones	>$10 billion	rDNA Immobilized Enzymes Fermentation
Human Medicine	Monoclonal Antibodies Antibiotics Hormones Steroids Diagnostics Blood Plasma Extenders	>$5 billion	rDNA Immobilized Cells Immobilized Enzymes Fermentation
Animal Husbandry	Growth Accelerators hormones pituitary gland stimulants Immunization passive enteric active	>$1 billion	rDNA Immobilized Cells Fermentation Peptide Synthesis
Aquaculture	Shellfish, Crustaceans, Finfish growth accelerators antibiotics live feeds	>$500,000	rDNA Cell Fusion Fermentation
Food & Feed Ingredients	Single Cell Protein Amino Acids Enzymes Vitamins	>$2 billion	rDNA Immobilized Enzymes Fermentation

production through fermentation of sugars and starch to ethanol and the fermentation of sugars and starch, or alcohols, to high protein feed and food ingredients.

One conservative way to lay the foundation for an industry based on genetically modified organisms is to start first with a fermentation process to make ethanol or high protein feed and food ingredients. Once the equipment and techniques for growing the organisms and work-up of the products are accomplished, it should not be difficult to buy, or even create, strains of these organisms which have been endowed by genetic engineering with the capacity to give higher yields or to synthesize high value products.

For example, Phillips has developed a continuous fermentation process with a proprietary organism which will ferment methanol into a high protein food product at high cell density, suitable for animal or human consumption. For countries with high internal production of carbohydrate including celluloses and lignin, this process offers a welcome source of internally produced protein.

Other companies which have developed single cell protein processes include Imperial Chemical Industry (ICI) in Great Britain, Hoechst in Germany, Mobil in the U.S.A., and Mitsubishi Petrochemical in Japan.

For the most part fermentation processes such as these use organisms which have been developed by classical genetics. They are well behaved organisms which can be grown dependably under well defined conditions. These organisms are, therefore, ideal candidates for genetic engineering modification to endow them with the capacity to make ethanol more abundantly, or a more nutritious single cell protein for food or feed ingredients, or specialty products such as human insulin, enzymes such as rennin, vaccines such as that recently developed by Genetech, International Minerals and Chemical (IMC) and the United States Department of Agriculture against foot and mouth disease, or specialty chemicals including biodegradable polymers such as the ICI polyhydroxybutyrate (PHB).

In regard to the genetic engineering of organisms, be they bacteria, plant, or animal, one must be aware not only of objectives but of side effects which may limit the use of the organism. The following two strategies illustrate this point.

One strategy is to cause an organism to initiate or increase production of a substance valuable to other organisms or man, but not essential to the survival of the organism itself. Increasing the capacity of a *Rhizobium* or *Pseudomonas* species to fix nitrogen is an example. Another, is to create a sugarcane plant with a higher yield of sucrose. Production of additional fixed nitrogen or sugar is energy-costly to the organism, hence, weakens its ability to survive. If the organism is grown under protected conditions, there is no problem. However, if the organism must compete with unmodified strains, for example, the *Rhizobium* or *Pseudomonas* in field soils or cane in fields adjacent to normal cane, the modified organism likely would be unable to compete.

The other, opposite strategy is to endow the organism with better survival characteristics. Examples are superior disease and insect resistance, and resistance to herbicides so that fields can be freed of weeds without harming the crop plants.

Agrigenetics is one leading company in the United States in this field of agricultural biotechnology. Cetus, under grants from two U.S. universities is moving toward transferring functioning genes into plant cells that will successfully grow into adult plants. Monsanto, Max-Plank Institute, and the University of Ghent are also working toward this end. Calgene, in California, is working on herbicide resistance in plants. Cotton is their first target crop.

This is a perfect example of application of genetic engineering principles to a crop of major importance in the United States but of little importance in many of the Central and South American and Caribbean countries. Incorporation of such resistance into coffee would, for Costa Rica, Columbia and Brazil, be more to the point. Such applications of this technology probably will not be initated in the near future unless carried out or commissioned by these countries.

Biotechnology can benefit Central and South American and Caribbean countries, but timing is of the essence. Benefits can be quickly realized and now is the time to move into the field.

BIOTECHNOLOGY AND AGRICULTURE: IMPORTANCE FOR THE DEVELOPING COUNTRIES

Glenn B. Collins

Department of Agronomy
University of Kentucky
Lexington, Kentucky 40546 U.S.A.

Technological developments made during the past decade in the fields of cellular and molecular genetics have been numerous and far-ranging as is quickly established from the burgeoning scientific literature. No area has been more active and rapidly advancing than the field of genetics where the status of knowledge and methods can best be described as explosive. The potentials for applying the newly gained genetic knowledge and manipulative techniques are so enormous that we have difficulty in assessing their impact on society. We know that cellular and molecular genetic methods can be coupled with conventional genetic and cytogenetic methods to produce improvements in microorganisms, plants, lower animals and even human beings. The objective of this paper is to assess the status of plant tissue and cell culture methods for economic plant species and to evaluate the potential uses of these methods in conjunction with conventional plant breeding for the improvement of economically important plant species in developing countries.

Any assessment of the potential applications of new biotechnological techniques such as tissue and cell culture must be based on two considerations: (i) the problem to be solved for the specific crop or species must first be identified, and (ii) then one must evaluate the possible use of tissue and cell culture as an aid and supplement to conventional breeding and genetic approaches to improving the specific crop or species. The crops, farming systems, and production problems encountered differ by countries and even regions within a country. Therefore, individual crop improvement needs and approaches to their solution must be considered.

PLANT TISSUE AND CELL CULTURE METHODS

Although plant tissue culture research dates back to the turn of the century and the early work by Haberlandt (1902), the real impetus to modern plant tissue culture was provided by the pioneering efforts of White (1939), Gautheret (1939), and Nobécourt (1939) some forty years later. Modern plant tissue culture embodies many different procedures and systems for growing plant cells, tissues and organs in an *in vitro* environment. Ideally we would like to be able to reduce every plant species to the single cell level for *in vitro* manipulations with the capability of subsequently regenerating an entire plant from that cell. Regeneration of plants from the single cell level is possible at present from a relatively small number of plant species, but the list is being expanded rapidly. The number of plant species for which tissues, organs and shoot tips can be grown *in vitro* with whole plant recovery is already quite extensive and impressive (Conger, 1981). Excellent progress is being made with the recalcitrant woody species (Bonga and Durzan, 1982) and the large seeded legumes (Phillips and Collins, 1981).

The rapid development of plant tissue and cell culture methods is also indicated by the series of recently published papers in an issue of Science. In this issue, Chaleff (1983) reviews the use of plant cell cultures for mutant isolation; Shepard et al. (1983) summarize the progress with protoplasts and protoplast fusion; Barton and Brill (1983) identify the use of cellular systems in genetic engineering; and Borlaug (1983) puts the interfacing of conventional plant breeding and newer biotechnological approaches into perspective. The ongoing plant tissue and cell culture research covers a wide range of economic crop species with the results being published in a very diverse set of journals and books. Although the focus for this meeting is directed more toward the tropical crops, the tissue and cell culture systems have been developed in large part without regard to where a crop is grown. The point made earlier is still valid, i.e., the necessity of first identifying the problem we want to solve and then determining if the *in vitro* or other biotechnological methods are available to use in solving the problem.

There has been rather intensive research activity in the tissue and cell culture area with crops of importance in the tropics. Three recent publications document this research and progress. I have already mentioned the use of tissue culture with woody species and the recent review volume edited by Bonga and Durzan (1982). The review by Mott in Conger's compilation (1981) also details progress made with woody species. A very recent publication edited by Rao (1982) contains a large collection of papers presented at a 1981 symposium in Singapore on tropical crop tissue culture covering a wide range of tropical crops. Although not exclusively devoted to tropical species, the proceedings of the 1982 International Plant Tissue and Cell Culture Congress convened in Japan contains a large number of papers detailing research progress on species of tropical importance (Fujiwara, 1982). Problems, approaches and limitations to biotechnology applications in Third World Countries were addressed very realistically in a recent paper by Swaminathan (1982).

AREAS OF APPLICATION OF TISSUE AND CELL CULTURE IN CROP IMPROVEMENT.

Complete tissue and cell culture systems providing the capability of growing cells and tissues from various explant source tissues and genotypes of a species are available for only a limited number of plant species. The requirements for culturing organized structures such as meristem and shoot tips as well as other organized structures can usually be readily defined and adapted for almost any species. *In vitro* manipulations of plants for improvement purposes can be viewed as involving different types of cultural methods and any available procedure for a species can be utilized even if other methods are not available for the species. In fact, utilization and development of one procedure for a species often leads to the development of other methods for that species.

Clonal propagation

The most readily accessible and useful tissue culture tool for plant improvement in developing countries is clonal propagation. This technique is generally easy to develop for any species and simply involves the *in vitro* multiplication of a large number of copies or clones of the initial explant. The need to proliferate large numbers of clones of a specific genotype is frequent in a breeding program. Clonal propagation is most useful in vegetatively propagated species where sexual multiplication is not available. The multiplication of sexually sterile lines, elite germplasm available in low quantity and self-incompatible lines are other examples of applications of clonal propagation. Major effort has been directed to the propagation of perennial woody species with long generation times. Iyer (1982) has identified numerous applications of clonal propagation with woody tropical species such as the palms. The vast opportunitites for utilizing *in vitro* clonal propagation are highlighted in the proceedings of the 1982 workshop focusing on biotechnology in international development reported in the Workshop Proceedings (1982) and in the working paper on plant tissue and cell culture by Collins (1982).

Disease elimination by meristem or shoot-tip culture

The commercial importance of producing virus-free plant stock is of considerable magnitude. The technique involves isolation of meristem tips from virus-infected plants and propagating many shoots and plants from a single meristem. The method is often facilitated by the use of a heat treatment. Younger and very small meristems often do not contain the virus and therefore virus-free plants can be produced from virus-infected stock for these species. Virus elimination is often carried out simultaneously with the multiplication of commercial material by clonal propagation.

The meristem and shoot culture technique is being used for a large number of ornamental species, fruit crops, vegetables and some agronomic species. The technique was developed for production of virus-free orchids by Morel (1960), and the applications for ornamental species remain extensive. Recent reviews by Lane (1982) and Harney (1982) identify disease elimination efforts in woody and herbaceous horticultural species, respectively. Virus elimination is also important in self-incompatible agronomic species where selected lines must be maintained for long periods of time by vegetative means and virus infection is a major problem. We have utilized meristem-tip culture to eliminate viruses from red clover germplasm (Phillips and Collins, 1979).

Tissue culture methods have been utilized to facilitate propagation and virus elimination in a number of tropical species including both herbaceous and woody species. In a review, Iyer (1982) has identified many responsive species including, for example, coconut, oil palm, date palm, *Citrus* species, tumeric, potato, ginger, papaya and sweet potato. Less spectacular but encouraging results have been obtained with coffee, tea, cacao and numerous other species.

Germplasm storage and interchange in tissue culture forms

Space and disease constraints restrict the number and diversity of germplasm accessions which can be collected and maintained for commercially important species and their relatives. The problems of bulky size, disease contamination, and viability are impediments to exchange of germplasm between scientists in different countries, even if the germplasm exists in collections or in wild habitats.

Storage of germplasm as meristem-tip cultures has been greatly facilitated by the development of cryopreservation methods for storing such materials. Kartha (1982) has recently reviewed the use of *in vitro* techniques for the preservation of germplasm including important tropical species. A useful feature of his review is a tabular list of species subjected to *in vitro* preservation procedures and references to the work.

The maintenance of a diverse germplasm base for economically important plant species and the interchange of disease-free germplasm are two essential needs in plant improvement. *In vitro* clonal propagation techniques are readily available or can be developed for most plant species. The application of these methods can have a major impact on crop improvement when used for mass propagation, disease elimination, germplasm storage and germplasm exchange.

Useful mutants from cell culture

Two powerful approaches to the isolation of potentially useful mutants from various types of *in vitro* plant cultures have been explored in recent years. The positive selection of mutants from callus, cell or protoplast populations of various economic plant species is based on the availabilty of millions of plant cells in a culture vessel and an extensive record of successful isolation of mutants from cultures of microorganisms. There is no question that exposing millions of cells to a selection agent under controlled conditions represents a powerful genetic tool. In addition, such a mutant can be isolated in an otherwise acceptable commercial cultivar genetic background for immediate practical use after appropriate performance testing. Overenthusiastic proponents of somatic cell selection approaches to crop improvement sometimes fail to consider fully the problems of identifying a suitable selection agent; regenerating a plant from the selected cell; establishing a genetic basis for the variant selected; and demonstrating stability and transmission through the sexual cycle. Excellent realistic reviews on *in vitro* selection have been written by Chaleff (1981) and Tomes and Swanson (1982). The most impressive variants selected to date include those conditioning amino-acid overproduction, disease resistance and herbicide tolerance and those tolerant to environmental stresses such as salt concentration and heavy metals. For species where the cell to plant regeneration step is possible, cellular selection offers a major opportunity for crop improvement.

The second source of potentially useful mutants from tissue and cell cultures are those which arise as an inherent consequence of the culturing process. There is a mounting body of literature which provides evidence that variants and mutants are either generated in tissue culture or are somehow revealed in plants regenerated from a variety of types of cell cultures in a number of different plant species. Numerous examples of such variability generated from tissue culture and termed somaclonal variation have been reviewed by Larkin and Scowcroft (1981). One of the most extensive investigations of variability exploitation from *in vitro* culture is the protoclonal variability observed in plants regenerated from protoplasts of a widely grown cultivar of potato by Shepard and his colleagues (1980).

Isolation of mutants by either positive selection or by capitalizing on somaclonal or protoclonal sources can be pursued in any species where plants can be regenerated from *in vitro* cultures. A well documented example of crop improvement utilizing such approaches is recorded for sugar cane by Heitz et al. (1977).

In vitro production of haploids and doubled haploids

The interest in and applications for haploid cells and plants in selection experiments and for the production of homozygous inbred lines in one step by doubling the chromosome numbers of a haploid individual have been greatly stimulated by the discovery of the anther and pollen culture methods for haploid plant production. We have recently reviewed the progress with anther cultures and the production of haploids in crop plants (Collins and Genovesi, 1982). The updated list of species for which anther or pollen culture has been utilized for the production of haploid plants demonstrates that the technique can be utilized for a large number of plant species including several woody species.

Other methods also exist for the production of haploids especially for those species where the anther culture method is inefficient. The production of barley haploids is accomplished by the pollination of barley with *Hordeum bulbosum* pollen and rescuing the interspecific hybrids by *in vitro* embryo culture. The *bulbosum* chromosomes are eliminated from the developing embryo and a haploid barley plant is recovered (Kasha and Kao, 1970). There are several species in which wide crosses between two species result in the parthenogenetic development of an unfertilized egg cell into a maternal haploid plant. We have recently observed the production of *Nicotiana* and *Petunia* haploids when either unfertilized ovules are cultured or ovules pollinated with pollen from another species are cultured (J. DeVerna and G.B. Collins, unpublished). A timely review on the use of unpollinated ovaries and ovules cultured *in vitro* for the production of haploids has been published by Yang and Zhou (1982). Additional systems for haploid production include spontaneous origin, semigamy as in cotton and the indeterminate gametophyte gene in corn.

The most direct and potentially significant contribution of haploids in plant improvement is for the rapid production of totally homozygous inbred lines. Inbred lines can be used as varietal materials in inbred species or for the production of F_1 hybrids in outcrossing species. Haploid breeding methods are being used in rice, corn, wheat, tobacco, and barley.

Haploids can serve as less genetically complex materials for cellular selection experiments, for genetic analysis and in the production of cytogenetic stocks.

Wide hybridization and gene transfer

The limits to the transfer of genes and chromosomes between plant species are rather restrictive both in nature and in the hands of the plant breeder. Numerous mechanisms have evolved to prevent interspecific and intergeneric hybridization among plant species. Nevertheless, many important and useful genes are known to be present in wild progenitor species and in distantly related species outside of the combinations which can be hy-

bridized sexually. Two recent developments have vastly expanded the transfer of genes between plant species. One is the improvement of tissue and cell culture methods for commercially important plant species and their wild relatives. This has enabled plant geneticists to apply the procedures of *in vitro* pollination/fertilization and *in vitro* hybrid embryo rescue to overcome numerous cases of prezygotic and postzygotic interspecific fertilization barriers, respectively. The methods of *in vitro* pollination/fertilization have been reviewed by Rangaswamy (1977), and Raghavan (1980) has reviewed embryo culture methods. A recent significant demonstration of the embryo rescue procedure is provided by the successful production of a perennial interspecific hybrid in *Trifolium* by Phillips et al. (1982).

The second development has extended the range of gene transfer dramatically and enabled the hybridization of very distant or even unrelated species. The method is somatic cell hybridization in which the sexual process is bypassed entirely through the fusion of wall-less cells called protoplasts. Rapid developments in this field have led to numerous successful interspecific and intergeneric hybridizations. An excellent review of methods and success with all plant species is provided by Keller et al. (1982). A review which is restricted to cereals and grains but covers all aspects of *in vitro* culture including protoplasts and somatic hybridization was also recently published (Vasil, 1982). Liberation of protoplasts from leaf, root and suspension cultured cells through treatment with a combination of cell wall degrading enzymes can now be routinely done for a vast array of plant species. Protoplasts from different species can be induced to fuse and form heterokaryons under reasonably well defined environmental and media conditions. The major limitation to gene transfer between species through somatic cell fusion is the general inability to regenerate entire plants from the single protoplast or heterokaryon stage. Other problems include identification and selection of the heterokaryons, stability of organelle complements and the stability of chromosomes from two widely divergent species contained in the same nucleus.

It is apparent that many technical obstacles must be resolved before somatic cell fusion will be useful as a general tool for gene transfer between distantly related species. Progress in this area is steady, and shorter term applications may include hybridization between more closely related species to yield polyploid hybrids or the transfer of cytoplasmic traits such as male sterility.

Production of chemicals by cultured plant cells

A large number of important chemical compounds used in flavoring, therapeutic, insecticidal, coloring, hallucinogenic and other roles for human benefit are produced by plants and identified generally as secondary products. An impressive list of such compounds and the Brazilian species producing them has been compiled by Crocomo et al. (1981). There has been a continuing interest and considerable research devoted to the production of useful secondary metabolites by cell cultures. The advantages of a contamination-free culture and the controlled growth conditions provided by the cell culture system may be offset by a reduced yield or lowered quality of the *in vitro* produced product. Since the yield, quality and stability of the secondary metabolite are influenced by the culture medium, culture system and culture environment, the development of *in vitro* production systems must be optimized for each species and product. The commercial feasibility of *in vitro* production of a given product must be determined by comparison to the *in vivo* production system.

In vitro systems for production of secondary products appear to be uniquely suited to situations where endangered species are involved. Other special situations may also warrant development of *in vitro* systems for producing valuable secondary products.

BRIDGING MOLECULAR GENETICS TO WHOLE PLANT IMPROVEMENT

Although recombinant DNA techniques and related molecular genetic approaches to crop improvement are still in the developmental stages, the strategies being employed for transferring genes into plants from exotic sources involve the use of cultured cells and tissues. Examples to date include studies with direct uptake of DNA by plant cells; transfer of DNA to plant cells in lipid membrane vesicles (liposomes); microinjection of DNA into plant cells; and culturing bacterial and plant cells together to transfer plasmid vectored genes. Viruses have also been introduced into cultured plant cells.

The potential role of cultured plant cells and tissues in utilizing the powerful tools of molecular genetics in the future should lend support to the development of improved and complete *in vitro* systems for important economic plant species. The efficient regeneration of complete plants from the single cell or protoplast level is a key and critical step to be accomplished.

SUMMARY

Major advances have been made in the development and application of cellular and molecular genetic techniques in plant improvement. These techniques are logically viewed as adjunct tools to be used as aids to plant breeding and other conventional approaches to crop improvement.

The progress has been noteworthy in the development and refinement of plant tissue and cell culture methods for important crop species. Usable applications must be ascertained for each plant species since complete *in vitro* systems which include all forms of tissue and cell cultures and the capability to regenerate plants from single cells are possible only for certain species.

Clonal propagation for plant multiplication purposes; meristem-tip culture for disease elimination; *in vitro* facilitated wide hybridization; and, germplasm storage and exchange via tissue cultures can presently be successfully accomplished for a large number of species in several different taxonomic groups of plants. Haploids and homozygous diploid lines derived from haploids can be generated with the aid of *in vitro* manipulations in a number of economically important plant species. Although the failure to achieve single cell to whole plant regeneration in many plants limits the application of cellular selection for desirable mutants and the recovery of somaclonal and protoclonal variants, this capability is being exploited for several plant species. Additional roles of a longer term nature can be envisioned for plant cells in culture in the production of important secondary metabolites and as a bridging mechanism for the transfer of genes through molecular genetic procedures.

A caution relative to considering the commitment of resouces and effort to the development and utilizaiton of plant tissue and cell methods for crop improvement is warranted. Employment of tissue and cell culture methods should be determined by evaluation that a specific problem can best be resolved most efficiently by their utilization.

REFERENCES

Barton, K.A., and W.J. Brill. 1983. Prospects in plant genetic engineering. Science 219:671-676.

Bonga, J.M., and O.J. Durzan. (eds) 1982. Tissue Culture in Forestry. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, Netherlands.

Borlaug, N.E. 1983. Contributions of conventional plant breeding to food production. Science 219:689-693.

Chaleff, R.S. 1980. Genetics of Higher Plants. Applications of Cell Culture. Cambridge University Press, Cambridge, England.

Chaleff, R.S. 1983. Isolation of agronomically useful mutants from plant cell cultures. Science 219:676-682.

Collins, G.B. 1982. Plant tissue and cell culture, an overview. pp. 230-253. In: Priorities in Biotechnology Research for International Development — Proceedings of a Workshop. July 26-30, 1982. National Academy Press, Washington, D.C.

Collins, G.B., and A.D. Genovesi. 1982. Anther culture and its application to crop improvement. pp. 1-25. In: Application of Plant Cell and Tissue Culture to Agriculture and Industry. Eds. D.T. Tomes, B.E. Ellis, P.M. Harney, K.J. Kasha and R.L. Peterson. University of Guelph Press, Guelph, Canada, 1982.

Conger, B.V. ed. 1981. Cloning Agricultural Plants Via In Vitro Techniques. CRC Press, Boca Raton, Florida.

Crocomo, O.J., E. Aquarone and O.R. Gottlieb. 1981. Biosynthesis of secondary products *in vitro*. pp. 359-372. In: Plant Tissue Culture — Methods and Application in Agriculture. Ed. T.A. Thorpe. Academic Press, New York, New York.

Fujiwara, A. (ed.). 1982, Plant Tissue Culture 1982. Proceedings of the 5th International Congress on Plant Tissue and Cell Culture, Tokyo and Lake Yamanaka, Japan, July 11-16, 1982. Maruzen Co., Ltd. Tokyo, Japan.

Gautheret, R.J. 1939. Sur la possibilité de réaliser la culture indéfinie de tissu de tubercules de carroti. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences 208:118-121.

Haberlandt, G. 1902. Kultureversuche mit isolierten pflanzellen. Akademie de Wissenschaften (Vienna, Austria) 111:69-92.

Harney, P.M. 1982. Tissue culture propagation of some herbaceous horticultural plants. pp. 187-208. In: Application of Plant Cell and Tissue Culture to Agriculture and Industry. Eds. D.T. Tomes, B.E. Ellis, P.M. Harney, K.J. Kasha and R.L. Peterson. University of Guelph Press, Guelph, Canada.

Heinz, D.J., M. Krishnaumutthi, L.G. Nickell, and A. Maretzki. 1977. Cell, tissue and organ culture in sugar cane improvement. pp. 3-17. In. Applied and Fundamental Aspects of Plant, Cell, Tissue and Organ Culture. Ed. J. Reinert and Y.P.S. Bajaj, Springer-Verlag, Berlin, Germany and New York, New York.

Iyer, R.D. 1982. Embryo and tissue culture for crop improvement, especially of perennials, germplasm conservation and exchange — relevance to developing countries. pp. 219-230. In. Tissue Culture of Economically Important Plants. Proceedings of an International Symposium, Singapore April 28-30, 1981. Ed. A.N. Rao. COSTED and ANBS. Singapore.

Kartha, K.K. 1982. Cryopreservation of germplasm using meristem and tissue culture. pp. 139-161. In: Application of Plant Cell and Tissue Culture to Agriculture and Industry. Eds. D.T. Tomes, B.E. Ellis, P.M. Harney, K.J. Kasha and R.L. Peterson. University of Guelph Press, Guelph, Canada.

Kasha, K.J., and K.N. Kao. 1970. High frequency of haploid production in barley (*Hordeum vulgare L.*). Nature 225:874-875.

Keller, W.A., G. Setterfield, G. Douglas, S. Gleddie and C. Nakamura. 1982. Production, characterization and utilization of somatic hybrids of higher plants. pp. 81-114. In: Application of Plant Cell and Tissue Culture to Agriculture and Industry. Eds. D.T. Tomes, B.E. Ellis, P.M. Harney, K.J. Kasha and R.L. Peterson. University of Guelph Press, Guelph, Canada.

Lane, W.D. 1982. Tissue culture and *in vitro* propagation of deciduous fruit and nut species. pp. 163-186. In: Application of Plant Cell and Tissue Culture to Agriculture and Industry. Eds. D.T. Tomes, B.E. Ellis, P.M. Harney, K.J. Kasha and R.L. Peterson. University of Guelph Press, Guelph, Canada.

Larkin, P.J., and W.R. Scowcroft. 1981. Somaclonal variation — a novel source of variability from cell cultures for crop improvement. Theoretical and Applied Genetics 60:197-214.

Morel, G. 1960. Producing virus-free *Cymbidiums*. American Orchid Society Bulletin 29:495-497.

Nobécourt, P. 1939. Sur la perénnite et l'augmentation de volume des cultures des tissus végétaux. Comptes Rendus des Séances de la Société de Biologie et de Ses Filiales 130:1270-1271.

Phillips, G.C., and G.B. Collins. 1979. Virus symptom-free plants of red clover using meristem culture. Crop Science 19:213-216.

Phillips, G.C., and G.B. Collins. 1981. Induction and development of somatic embryos from cell suspension cultures of soybean. Plant Cell, Tissue, and Organ Culture 1:123-129.

Phillips, G.C., and G.B. Collins and N.L. Taylor. 1982. Interspecific hybridization of red clover (*Trifolium pratense L.*) with *T. sarosiense Hazsl.* using *in vitro* embryo rescue. Theoretical and Applied Genetics 62:17-24.

Priorities in Biotechnology Research for International Development — Proceedings of a Workshop. July 26-30, 1982. National Academy Press, Washington, D.C.

Raghavan, V. 1980. Embryo culture. International Review of Cytology, Supplement 11B. 21:209-240.

Rangaswamy, N.S. 1977. Application of *in vitro* pollination and *in vitro* fertilization. pp. 412-425. In: Applied and Fundamental Aspects of Plant Tissue, Cell and Organ Culture. Eds. J. Reinert and Y.P. S. Bajaj. Springer-Verlag, Berlin, Germany.

Rao, A.N. (ed.) 1982. Tissue Culture of Economically Important Plants. Proceedings of an International Symposium. Singapore April 28-30, 1981. COSTED and ANBS. Singapore.

Shepard, J.F., D. Bidney and E. Shakin. 1980. Potato protoplasts in crop improvement. Science 208:17-24.

Shepard, J.F., D. Bidney, T. Barsby and R. Kemble. 1983. Genetic transfer in plants through interspecific protoplast fusion. Science 219:683-688.

Swaminathan, M.S. 1982. Biotechnology research and third world agriculture. Science 218:967-972.

Tomes, D.T., and E.B. Swanson. 1982. Application of *in vitro* selection to plant improvement. pp. 25-43. In: Application of Plant Cell and Tissue Culture to Agriculture and Industry. Eds. D.T. Tomes, B.E. Ellis, P.M. Harney, K.J. Kasha and R.L. Peterson. University of Guelph Press, Guelph, Canada.

Vasil, I.K. 1982. Plant cell culture and somatic cell genetics of cereals and grasses. pp. 179-203. In: Plant Improvement and Somatic Cell Genetics. Eds. I.K. Vasil, W.R. Scowcroft and K.J. Frey. Academic Press, New York, New York.

White, P.R. 1939. Potentially unlimited growth of excised plant callus in an artificial nutrient. American Journal of Botany 26:59-64.

Yang, H.Y., and C. Zhou. 1982. *In vitro* induction of haploid plants from unpollinated ovaries and ovules. Theoretical and Applied Genetics 63:97-104.

BIOTECHNOLOGY AND ANIMAL HEALTH

Dennis G. Howell

Ontario Veterinary College
University of Guelph,
Guelph, Ontario, Canada

This paper concerns the application of biotechnology to animal health. In so far as developing countries are concerned, we must look at the matter in the wider context of the total delivery of veterinary services. By this, I imply we must look at the matter from an animal production standpoint and not solely against the narrower field of disease control and prevention. Those of us who are concerned with such matters view animal production and animal health as closely intertwined, and in fact, most schools of veterinary medicine in developing countries educate veterinarians from this philosophical standpoint and produce animal production scientists as distinct from the North American standpoint of the animal doctor. Mind you, even our North American schools are seeing the logic behind such a philosophy, and most curricular changes have this objective in mind. The present topic is not, however, the education of the veterinarian, but I feel so strongly on this point that I feel compelled to make it.

In looking at the application of biotechnology, we must remember that animal production, whether it is in the industrialized north or the developing south, must be looked at from a systems standpoint and recognize that it involves a spectrum of disciplinary inputs — a number of which lend themselves to biotechnological advance. In simple terms, the system consists of genetics as a starting point, progressing through breeding and reproduction to nutrition, to health and finally, to marketing. Biotechnology, if we apply the customary definition of the use of biological resources whether they be microbial, plant, or animal for the production of goods and services, clearly has application to the first four of these disciplinary inputs.

It must be conceded that, at least in terms of the current state of the art, it is in the areas of health and nutrition that biotechnology is having its greatest impact. However, if we consider artifical insemination and ovum transplantation as applications of biotechnology, as many properly do, then of course we should give a little more than a passing mention to it. The ability to preserve both sperm and ova of highly productive breeds of livestock and, indeed, to preserve pools of many exotic breeds which may have later application is a priority matter to both FAO and the United Nations Environmental Program. However, since the present focus is primarily on microbiology, I will forego dealing with that aspect here. I will concentrate on firstly, health, secondly, nutrition and finally, remarks on the possible implication on genetics which the recent reported work on giant mice may have.

DISEASE CONTROL

The control of animal disease by means of vaccines and immune sera, developed from the microorganism responsible for the disease in question, has been a prominent feature of veterinary medical practice for the past hundred years. Indeed, many of the major scourges which have plagued animal farmers since earliest recorded times have been brought under control by methodologies of this kind: anthrax, rinderpest, foot and mouth disease, hog cholera and Newcastle Disease of poultry to name only a few. Veterinary medicine lends itself particularly to such procedures. Insofar as food animals are concerned, the problem resides in the herd or flock rather than in the individual animal, and control of disease on a herd or flock basis is of paramount importance to the national well-being both in developed and developing countries. The use of living agents as vaccines has been particularly useful in the control of animal diseases. These have varied from naturally occurring microorganisms to artificially attenuated strains. With all this, there is, however, a constant thought regarding their safety, the possibility of reversion, and the establishment of reservoirs of infection. The fact remains that in most instances the level of immunity produced by a live vaccine is so much better than that produced by a comparable killed vaccine that their use is advocated whenever possible. Furthermore, the frequency of vaccination with live vaccines is usually less, a considerable item to take into account. An additional factor of importance is that some of these vaccines can be given other than by injection. In intensively reared poultry flocks, for example, the fact that vaccines against Newcastle Disease or infectious bronchitis can be given in the drinking water is a matter of considerable importance. If every individual animal in an operation of some thousands has to be individually innoculated, there is a considerable cost element, and indeed, there will inevitably be a number of fatalities associated with penning and handling. All this creates a scenario for the acceptability of genetically engineered microorganisms that induce high quality immunity, whose method of administration is practical, and with which there is no problem of safety.

Infectious diseases of livestock are caused by viruses, bacteria, protozoa and a variety of internal and external parasites. In some instances, the latter act as vectors for the former. The fact that in many instances bacteria, pro-

tozoa and parasites can be readily controlled by chemo-therapy and chemo-prophylaxis makes the possibilities of biotechnology and genetic engineering less exciting than it does for viral diseases. In almost every instance, the control of the latter depends on the availability of effective vaccines. While the door is only just beginning to open as to the possibility of genetically engineered viral vaccines, one can predict with some confidence that the future will indeed be exciting. The advantages are so great that one must applaud the pioneering research that is currently underway in many places and encourage national and international funding agencies to give high priority to programs of this kind. Some of the advantages that readily spring to mind are:

1. The antigen required to create immunity is isolated from the rest of the microorganism and, as such, is non-disease producing. This fact alone presents enormous advantages with regard to safety in the manufacturing process.
2. With many conventional inactivated vaccines, the chemical processes required to render them innocuous reduce their protective immunogenicity considerably. A genetically engineered vaccine requires no inactivation procedure.
3. The possibilities of producing a genetically engineered viral vaccine through the medium of bacteria considerably reduces production costs. Expensive procedures involving, for example, mammalian cell tissue culture systems, are replaced by a relatively simple conventional bacterial medium.
4. Additional economics in production will arise from the savings on high security plant. The costs of producing conventional foot and mouth disease vaccines are considerable because of the high security buildings required.
5. Safety testing on a large scale will be avoided since the vaccine does not contain the disease-producing entity.
6. Transportation and storage costs will be less as a result of reduced requirements for refrigeration, freeze drying, etc.

I propose to review progress on vaccine development in a few diseases of major importance to veterinary medicine, particularly when viewed on a global scale, and to see where recent developments are likely to take us.

Foot and Mouth Disease

While a number of countries are free from foot and mouth disease, it still constitutes a very grave and serious problem in many others where it is endemic. These include some Eastern European countries, South America, and much of Asia. While it is not a killing disease, its effects produce great economic loss, both directly and indirectly. Its existence in a country severely hampers the development of that country's international trade in animals and animal products.

Foot and mouth disease vaccines have existed for many years. The Frenkel inactivated vaccine prepared from material obtained from the lesions themselves has been extensively used for many years. Recent developments involving tissue culture material have resulted in the issue and use of many hundreds of millions of doses per annum. Usually a trivalent vaccine is employed, covering the main serological types although in point of fact seven types have now been identified. In the long history of the use of foot and mouth disease vaccine and in regard to the virulence of the virus, it is not surprising that a number of outbreaks have occurred due to escape of virus from the manufacturing laboratories or to inadequate inactivation. Events such as these serve as a sufficient stimulus for the production of a form of vaccine not bedevilled by hazardous problems of such kind.

During recent years, research on novel foot and mouth disease vaccines in a number of laboratories has been based on recombinant DNA and related techniques. Foot and mouth virus, which is an RNA virus, has had messenger RNA converted into DNA, and the nucleotide sequence of the immunogenic viral protein has been expressed in *Escherichia coli*. The resulting protein has demonstrable immunogenic potential when evaluated in animal experimentation. Those countries which enjoy the appropriate facilities for carrying out challenge experiments have been in the forefront of this research. Considerable advances have been made in joint studies in the U.S.A. by a commercial biotechnology company and the U.S. Department of Agriculture.

The synthetic peptide approach offers a further possible advance in the development of foot and mouth vaccines. By means of computer modelling, the molecular location of the determinant group may be approximately determined. Further refinement of the location may be by the use, for example, of monoclonal antibody which if it neutralizes the disease producing propensity of the virus will be strong confirmatory evidence that the determinant group has been properly located. Its amino acid sequence can thus be determined which thus permits synthesis to be carried out. There is, of course, the inevitable question as to the quality of vaccines developed by such an approach. At best, a peptide is a hapten, and there are those who consider the more direct recombinant DNA approach as likely to lead to better results.

Another possible approach involves the so called combined live-killed vaccinia-like vaccines. Essentially this involves the introduction of the required configuration into an otherwise relatively harmless virus. While I am not aware that this has been attempted with foot and mouth vaccine — an appropriate introduction from the

virus of Marek's disease has been made into avian pox virus. From an immunological point of view, the concept should have merit since, presumably, cell mediated immunity would be generated in addition to conventional circulating antibody.

Rabies

Rabies is a disease of considerable significance to man and animal alike. A recent assessment of the economic losses in cattle production due to bat-transmitted rabies in Latin America amounted to about $28 million per annum. Vaccines of various types are now being widely used. A number of laboratories are working on genetically engineered rabies vaccines. Such would have considerable advantage for cattle vaccination.

It is generally held that rabies virus is antigenically uniform, but recent monoclonal-antibody studies in the U.S.A. indicate this may not be so. If correct, antigenic variants could explain some instances of alleged vaccine failure.

A possibility of combining monoclonal antibody and interferon for dealing with those exposed to rabies might be useful in slowing down virus multiplication. This would obviously have greater application in man than in domesticated animals.

Newcastle Disease

Newcastle Disease of poultry is a matter of considerable economic loss, and vaccination is widely practiced in all parts of the world. While strains vary with regard to virulence, there is no evidence of significant antigenic variation. Several vaccines are used, both live and killed, but improvements are required both in potency and in geographical utility. Genetic engineering might afford a solution, particularly insofar as the production of massive quantities of antigen is concerned.

A short introductory workshop paper such as this does not permit describing a number of other possibilities insofar as genetically engineered vaccines are concerned. There are a number which might be worthy candidates for development. I am thinking perhaps of brucellosis, Aujesky's disease, blue tongue, African horse sickness, etc.

Before leaving the matter of immunization, mention has to be made of the possibility of feeding monoclonal antibody for protection against neonatal disease. These conditions are extremely important in calves and piglets. A great deal of research has been undertaken with regard to the passive immunization of the newborn.

NUTRITION

Single Cell Protein (SCP)

The use of microorganisms as food for animals is a matter which has been highly topical since the sixties. Many developments have taken place, but the concept is exceedingly straightforward. A suitable microorganism such as a yeast is cultured in carbohydrate media, and the biomass is then processed to produce a material which is rich in protein. A number of commercial undertakings have developed processes of this kind. Some even have found microorganisms that would grow in petroleum products and attempted to develop processes as a bi-product of the petroleum industry. Most of the developments have occurred in Europe. In the seventies, two UK commercial firms moved into commercial production; however, only one, ICI, has continued the development. The problems are two-fold. First of all, the use of petroleum products as the substrate could conceivably raise objections to carcass quality due to high levels of residual hydrocarbon in the meat. Secondly, the relatively and continuing low price of soya bean makes the competitive situation somewhat precarious.

With the growing disillusion over the use of petroleum-based products, attempts are being made to utilize alternative substrates. The use of waste features prominently in these considerations, particularly those wastes that arise as effluents in the food industry. Effluents that are rich in carbohydrate but free of possible toxic factors are clearly attractive. The milk and cheese industries clearly spring to mind in this context, as do confectionery manufacturers. Indeed, in the UK and in Sweden, a number of food manufacturers have developed processes of this kind. Brewery wastes also offer distinct possibilities. The bioconversion of lignocellulose affords useful possibilities for the breaking down of indigestible vegetable waste to material digestible by ruminants. In a number of countries, the breaking down of straw, for example, rice straw, into digestible fodder is a practical proposition.

Growth Hormone

Very recently, results of an intriguing series of experiments by a group of U.S.A. scientists have been published in Science*. Genetic engineering is involved here, and it involves the principle of transferring genes into animal cells. In the last three to four years, several reports have appeared telling of the successful transfer of genes from one cell type to another and even from one animal to another of a different species. However, until the report referred to above was published, it was questionable whether the transferred genes were functional

* *Palmiter, R.D., G. Norstedt, R.E. Gelinas, R.E. Hammer, and R.L. Brinster. 1983. Metallothione-human GH fusion genes stimulate growth of mice. Science 222:809.*

in any meaningful way. The report involves the fusion of a strong and active regulatory genetic element to a useful gene. Essentially what has been accomplished is that the gene that is responsible for the production of growth hormone by the pituitary gland of rats has been combined with that responsible for a strong metabolic process in mice. The fused complex has then been introduced into a fertilized mouse egg which in time was then placed in a surrogate mother. The resulting newborn mice, although normal at birth, continued to grow rapidly and achieve twice normal size. This discovery clearly raises interesting points of conjecture in relation to animal industry both in terms of the production of meat and in relation to other animal products such as milk and hormones. Clearly this work will command a great deal of interest since the possibilities are considerable for animal industry.

BIOTECHNOLOGY AND HUMAN HEALTH

William D. Sawyer

Dean, School of Medicine
Wright State University
Dayton, Ohio 45435 U.S.A.

Others have commented extensively on the economic potential of biotechnology. They have focused on bioconversion, on agriculture and on animal production. Although the topic of biotechnology and human health has a strong humanistic base, i.e., the physical well-being of the people, it also has significant economic implications. Health-care costs are rising rapidly throughout the world. Further, enormous economic value is derived from the full development of human potential. A few observations illustrate both humanistic and economic considerations: at least 25 percent of the world's population suffers hunger and malnutrition; half of the hospital beds in the world are occupied by people suffering water-borne diseases; and infectious diseases during infancy and early childhood may impede physical and mental growth. Each of these examples has major significance for developing countries. These and other factors combine to sap the intellectual and physical vigor of many nations with untold consequent economic loss. What can we expect biotechnology to contribute to the prevention of lost human potential, to the reduction of the cost of health care and to the improvement of the human condition?

In the developed countries, biotechnology has grown within the perspective of highly sophisticated research, e.g., the production of insulin, growth hormone, interferon and the like. The focus is often on diseases of a chronic nature, cancer or rare, but serious human disorders. Processes such as *in vitro* fertilization and the correction of genetic defects are subjects for the application of modern biology.

The developing countries generally have interests and disease problems similar to those of developed countries. The developing countries have, however, another set of problems. Many of the latter are diseases of populations as well as of individuals and are disorders related to the environment, e.g., malaria, amebiasis, diarrheal diseases, schistosomias and leptospirosis. For many of the countries, the well known approaches to environmental disease such as sewerage and waste treatment, clean water and vector control are presently limited by cost. As a supplement or an alternative, biotechnology can, I suggest, offer much if we have the skill and the will.

The developing countries cannot rely on the developed nations for biotechnological solutions to problems of local importance. This is especially true if the industrialized nations have nil to low self-interest either for economic potential or for health reasons. These are, after all, the major motivating forces for the application of biotechnology to problems of human health. Two examples illustrate. The current hepatitis B vaccine could be of significant benefit to residents of many developing countries wherein infection is prevalent. The cost is, however, possibly prohibitive. Although the developed world is utilizing biotechnology to pursue a cheaper vaccine, the cost of research and development still may preclude the developing nations taking advantage of the badly needed hepatitis B vaccine. In further illustration, progress toward a malaria vaccine has recently been slowed because of economic issues, especially the economic incentives for commercial development. It is too soon to know the eventual economics of the malaria vaccine and how they will impact on its adoption. In any case, the present debate will undoubtedly delay the availability of this vaccine that is so badly needed in many of the developing countries. For many of the problems of developing countries, therefore, it is important that the nations or regions develop strategies for the application of biotechnology within their area, at least in regard to solutions to problems that are not shared with the industrialized nations.

In considering some prospects for biotechnology, I will focus on a field that impacts large segments of the population in developing countries, infectious diseases.

Rapid diagnosis is important to the management of infectious diseases for it affords the opportunity for early specific therapy with consequent shortened hospital stay, quicker return to work and reduction of complications.

Earlier communications in this conference have emphasized that proteins can now be considered bulk chemicals. This offers great potential. Biotechnology should soon provide for the production of specific proteins of diverse infectious agents at reasonable cost, thus facilitating serological diagnostic tests.

A diagnostic area with high promise from biotechnology is the use of DNA probes for the specific identification of microorganisms. Such probes may be produced in bulk and used with relatively simple equipment. Presently, radioactive reagents are required. Innovative fluorescent chemical techniques give promise, however, of eliminating the need for radioisotopes and equipment for quantifying radioactivity.

Biotechnology offers another promising tool for improved diagnosis, monoclonal anitbodies. Monoclonal tech-

nology allows production in quantity of murine antibody of uniform specificity for use as diagnostic reagents. These reagents may be used in improved immunoassays and in serologic tests seeking to identify specific antigens in samples from patients with infectious disease.

In addition to the development of reagents, modern biological research has improved diagnostic techniques. Procedures such as ELISA, coagglutination utilizing staphylococcal protein A and immunofluorescence are now commonplace and are being exploited with great vigor in the diagnostic laboratory.

The application of biotechnology to the diagnosis of infectious diseases will revolutionize the diagnostic laboratory. The developing nations must be able to participate in the benefits of this revolution. In the developed countries, much emphasis is given to diagnostic techniques and equipment that reduce labor. Such techniques and equipment are usually capital intensive, but this is more than compensated by the reduction in the cost of labor. In developing countries, the opposite usually prevails. That is, whereas capital is in short supply, labor is typically both plentiful and reasonable in cost. There may be a shortage of highly skilled labor, but individuals can be trained for the application of labor-intensive diagnostic techniques. It is apparent, therefore, that the need in the developing countries is for the development of rapid diagnostic techniques that are not capital intensive. Such procedures may have to be developed within the region. Similar considerations apply to reagents. If the needs of developing countries for specific diagnostic reagents coincide with those of developed countries and if costs allow, technology transfer may suffice. Relatively unique requirements will, however, probably be met sooner through national or regional foci for the production of reagents that are mainly relevant locally. In addition, local production facilities for reagents and equipment, even though the production process originated elsewhere, may be advantageous. Regional cooperatives may prove cost effective for the developing nations.

Biotechnology can also help improve treatment, a second aspect of the management of infectious diseases. Others have considered how technology can enhance the production of therapeutic drugs and antibiotics. The intense search in the industrialized countries for improved antimicrobial agents will undoubtedly provide much progress. In my view, the developing countries might better utilize their resources otherwise.

Monoclonal antibodies can be developed in human systems. As this technology improves and is adapted to large scale, human antibodies may be of importance in therapy. For example, toxic moieties may be linked to monoclonal antibodies that will interact specifically with tumor cells. Thereby, a potent attack against tumors might spare normal host cells. Such reagents would have great potential for therapy of malignancy. In regard to infectious diseases, immunotherapy offers hope for diseases such as rabies and other serious viral infections, conditions for which a full spectrum of effective anti-viral drugs is not available. The developing countries have an outstanding opportunity to develop and utilize monoclonal antibodies as therapeutic agents for diseases that are commonplace there, but rarely seen in the developed nations.

Biotechnology's greatest potential for a direct contribution to human health in the developing countries lies, I believe, in the prevention of infectious diseases. Biotechnology can greatly enhance the development and the production of vaccines, a keystone of prevention. Although biomedical science has brought about notable successes in immunoprevention, e.g., diphtheria, tetanus, polio, measles and smallpox, the overall record of development of effective vaccines is weak. The greatest limitation has probably been that of developing the necessary pure antigens in quantity. The capacity of biotechnology for the production of antigens should soon provide a solution. However, the cost of research and development in relation to potential earnings will remain an issue that is likely to limit progress in the developed countries. It may, therefore, be important for developing countries to find creative ways to produce vaccines for their own needs. One example is the plan for the creation in the ASEAN countries of a vaccine development center for vaccines of regional importance. Other areas ought to watch carefully the progress of this center and consider like approaches.

How can the application of biotechnology to vaccine development be approached? A key first step is the development of national-regional priorities. Each area needs to assess its infectious problems and needs for vaccines in relation to a number of factors. This approach was illustrated in a recent workshop sponsored by the U.S. Naitonal Academy of Science. Among the criteria for new investment in vaccine development were: current vaccine status, feasibility, current funding for research, and public health significance. That conference assigned high "world" priority for new funding and a biotechnological approach to vaccines against: rabies, dengue, Japanese encephalitis, baterial respiratory diseases (especially, *Streptococcus pneumoniae*, hemophilus B and *Bordetella pertussis)*, baterial enteric diseases, chlamydia, malaria and leishmaniasis. Continued emphasis on other diseases is also needed, e.g., influenza and schistosomiasis. National or regional creation of a local priority list would facilitate the determination of the most cost-effective project(s) for application of resources.

Presently, I see several basic technical approaches to vaccine development. The first is a continuation of conventional procedures such as the isolation of toxins followed by toxoiding, the development of attenuated organisms and the isolation of immunogenic fractions that give rise to protective immunity. Biotechnological advances may facilitate production, particularly of toxoids and of protective fractions.

Among newer approaches is the use of recombinants as vaccine agents. Dr. Howe considered "live-killed" viral

vaccines, those in which the gene for the protective antigen of a pathogen is incorporated into the genome of a non-pathogenic virus. The recombinant is used as vaccine. Similar techniques have been applied to shigellae. Gene segments determining protective antigens have been incorporated into harmless *Escherichia coli*. The latter are then used as the living immunizing agent because of their ability to express, without risk, the protective antigen of shigellae. The full potential of this technology is yet to be determined.

Another technology is molecular cloning and the production of vaccine antigens by fermentation. This is essentially the bulk production of protein considered by others. The potential is limited only by the ability of specialists in microbiology, immunology and infectious disease to identify the antigens needed, the capacity of genetic engineers to clone the genes and the skill of biotechnologists to produce the antigen in bulk.

Another attractive prospect lies in the production of synthetic peptides that can be used in the construction of vaccine. This technology derives especially from the work of Richard Lerner and colleagues. The concept is simple. One first identifies the protein(s) of significance in the pathogenesis of infection and isolates the DNA coding for the proteins(s). From the DNA sequence, the amino acid sequence of the protein is predicted. Various peptide segments of the protein are synthesized *in vitro* and individually coupled to carrier molecules such as hemocyanin or a toxoid, e.g., tetanus toxoid. The synthetic peptide-protein complex is used as the immunizing agent. Studies show that resultant anitbody may react with the specific peptide and with both the original microbial antigen and the intact organism. Further, the appropriate synthetic-peptide segment coupled to form antigen can confer protective immunity. Promising results have already been achieved with foot and mouth disease virus and with influenza virus. In a related application of importance to the world efforts at the control of population, a synthetic peptide corresponding to the C-terminal portion of human chorionic gonadotrophin has been tested as a birth-control vaccine in sub-human primates. Results to date are promising. Consideration is being given to a phase-I human trial of this synthetic-peptide vaccine.

Each of these techniques has great promise. If the technology can be brought to the level of routine production for one infectious disease, the process will likely be broadly applicable to the many needed vaccines. That is, once the principal technology is in hand, it can be utilized again and again.

Although the application of biotechnology will improve human health, a number of problems remain if the developing countries are to participate fully in this technological revolution. Others have emphasized both some of the technical problems in the application of biotechnology and the need for multidisciplinary approaches and a critical mass of biotechnolgists for optimal achievement. I would add infrastructural needs to the list. Effective utilization of the technologies requires ready access to enzymes, to essential equipment, to reagents, to media, to a reliable source of power, etc.

In addition, there are organizational and institutional needs that must be met if biotechnologists and their organizations are to achieve their potential. Organizations as well as the individuals of which they are composed must be patient, persist and work diligently. Biotechnology organizations need endorsement, i.e., a commitment by government or by private sector agencies to science and technology as ways to solve important problems. This endorsement must be durable so that the institutions may survive temporary political or economic instability.

To be productive, I believe, an organization must have within it a set of human attributes. This is a collective requirement of the organization, one that will likely be found as a composite from several people rather than in a single individual. The organization must, therefore, learn to optimize the contribution of each of its members. I divide these attributes into five.

The first requirement within the organization is creativity and ingenuity. This is the scientific base and the technical base upon which productivity depends.

A second need is entrepreneurship as the term is used in the business community. It is a positive attribute. Entrepreneurs are individuals who can recognize the value and potential of an idea and gain the acceptance of those who must accept it whether for purchase or support. While creative scientists may be entrepreneurs, the combination is far from common.

The third need is managers — the people who bring together all of the parts so that products are produced efficiently and on time. Organizations should avoid the common error of assuming that creative individuals or entrepreneurs are inherently good managers.

A fourth need is people who can serve as the eyes and ears of the organization. These are individuals who are broadly knowledgeable, widely read and travelled, and good communicators. At meetings and when reading, they recognize ideas that may be of value to others in the organization and can effectively communicate those ideas. Such individuals must know the progress in the field and the regulations and the trends that will influence the overall operation. It is critical that they communicate to all important levels within the organization.

Finally, the organization needs people, generally senior, who can serve as promoters or sponsors of individuals within the organization. This is very important if internecine rivalries and competition are to be minimized and those energies devoted to the success of the operation *in toto*.

I urge that developing countries consider these organizational needs along with local traditions as they proceed with organization and institution building for biotechnology — or in other developmental efforts for that matter.

Biotechnology can contribute to improved human health. Such improvement will also enhance significantly the economic development of the lesser developed nations. If we in the biomedical sciences have the skill and the will, we can utilize the powerful tools of biotechnology to better the well-being of mankind throughout the world.

BIOCONVERSION OF LIGNOCELLULOSE

Carlos Rolz

Head, Applied Research Division
Instituto Centroamericano de Investigación y Tecnológia Industrial
Apartado Postal 1522, Zona 10
Guatemala City, Guatemala

In the EX-FERM process, ethanol is obtained by the extraction-fermentation of sucrose present in sugarcane by the action of suitable microorganisms (Rolz et al., 1983). In 1000 kg of cane, there are, on the average, around 130 kg of fermentable sugars and 125 kg of a fibrous residue. Usually, this last product is employed as fuel to purify the ethanol produced.

The hypothesis discussed here deals with the existing alternatives for employing this lignocellulosic residue, separating it into its major macrocomponents by a series of treatments and using the resulting products either as raw materials for producing more ethanol or as a source of energy for the overall process. A detailed energetic balance is beyond the present scope as very frequently new engineering schemes are presented that lower the energy demand to purify fermentation ethanol. What is important, however, is that, no matter how small, a fraction of this residue still must be used as process fuel.

The principal macrocomponents of the fibrous residue of cane are cellulose, lignin and hemicellulose. Their distribution varies with cane variety and age, agricultural practice and ecological conditions. A sample of sugarcane from the Pacific coast of Guatemala taken in the later part of the 82/83 crop was EX-FERmented; the lignocellulosic residue was dewatered and washed in a screw press and sun dried. Analysis according to techniques used with fibrous feeds or forages (van Soest and Robertson, 1980) yielded the following values: cellulose, 48%; lignin, 12%, and hemicellulose, 17%. The hemicellulose content was much lower than that in samples previously analyzed, which gave the following average results: 44, 15 and 40%, respectively. These data are within the values for sugarcane bagasse reported in the literature (INIP, 1983; Jackson, 1977; Randel, 1972; Rexen, 1979; Han et al., 1983, Ibrahim and Pearce, 1980, 1982). The *in vitro* dry matter digestibility (IVDMD) of such product is relatively low due to its lignin content and structural characteristics. However, the reported values vary widely, not only because of the factors mentioned above, but also due to analytical differences, for example, the use of rumen liquor or commercial enzymes to carry out the hydrolysis. In the same EX-FERmented sample, an IVDMD of 18.8% was determined (employing commercial enzymes). In the literature, values between 9.2 and 31.6% are found (Martin et al., 1974; Cabello et al., 1981; Dekker and Richards, 1972; Preston, 1975; Randel, 1972).

During the design of strategies to separate the macrocomponents of lignocellulosic materials for their further biotransformations, it is necessary to realize that their rates of reaction must be increased. Hence, pretreatment operations must be an integral part of any process scheme. It is well to remember that there is no optimum general pretreatment, as this will depend on the process, the products, raw materials and microorganisms to be used subsequently.

One interesting alternative in cases where ethanol is the desired final product is the delignification "Organosolv" process (Kleinert, 1974, 1975; Bowers and April, 1977; April et al., 1979; Baumeister and Edel, 1980; Katzen et al., 1980; Sarhanen, 1980; April et al., 1982; Green and Sawyer, 1982; Hansen and April, 1982; Marton and Graizow, 1982; Averignos and Wang, 1983; Neilson et al., 1983). Basically, lignin is separated by mixtures of ethanol-water acting above atmospheric pressure. Cellulose remains in the fibrous residue. Usually, hemicelluloses are hydrolyzed to pentoses. Lignin can be separated easily from the solvent. Ethanol (which is also the main product of the process) is the solvent rather than a chemical derived from fossil resources, like phenol. Phenol has been used successfully for the same purpose in the BATTELLE-Geneve process. Phenol can eventually be obtained from the pyrolysis of the separated lignin. The hemicellulose has been broken to fermentable sugars (pentoses), and cellulose, still as a polymer, has been transformed to a more reactive form. At ICAITI, experiments have started employing ethanol-water alkaline mixtures with and without the addition of anthraquinone. With ethanol-water (60:40) at 175° C for 6 h, a solids yield of 74% was obtained with a Kappa number between 129 and 139. By adding 2% NaOH and reducing the reaction time to 4 h, the results were 56% and 7, respectively. With anthraquinone, the values were 60% and 5.5, respectively.

The alkali action on lignocellulose causes a swelling of the solid matrix which allows solvents, chemical reagents or biological catalysts to penetrate further within and establish a much better contact with the internal structure. If the objective of the pretreatment does not require a lignin separation or a hemicellulose hydrolysis, an ade-

quate alternative is the solid phase alkaline process. In this case, the lignocellulosic residue arranged in a pile is sprayed with a concentrated alkaline solution, followed by a storage at ambient temperature, usually for one week. The doses are between 3-5 g/100 g of dry matter (Capper et al., 1977; Jackson, 1977; Dobie and Walker, 1977; Wilkinson and Gonzalez, 1978; Berger et al., 1979, 1980; Solaiman et al., 1979; Acock et al., 1979; Garret et al., 1979; Church and Champs, 1980; Willis et al., 1980; Lesoing et al., 1980; Berg and Mietz, 1980; Plasse et al., 1980; Benghedalia et al., 1980, 1981; Miron and Benghedalia, 1981; Chesson, 1981; Higgins, 1981; Jayasuriya and Perera, 1982). In this process, there are no effluents and very little dry matter is lost. In contrast, when the alkaline process employs suspensions, liquid effluents are produced. These need some kind of processing. The alkali effects are dramatic on the lignocellulosic residue, increasing substantially the potential degree of hydrolysis. With 12% NaOH on sugarcane bagasse, the IVDMD and enzymatic solubility increased by 582% and 280%, respectively (Cabello et al., 1981).

Sometimes, treatment of the solid residue with SO_2 is more effective than with alkali. Doses are around 5 g/100 g dry matter, followed by storage for three days at 70°C. Experimental data on wheat straw have been published by Ben-Ghedalia and Miron (1981).

Finally among solid state pretreatments, biological processes utilize either bacteria (Deschamps et al., 1981; Odier et al., 1981; Janshekar and Fiechter, 1982) or basidiomycetes (Sing and Rajarathnam, 1977; Zadrazil, 1977, 1978, 1979, 1980 a-d; Eger, 1978, 1979; Chang, 1978; Lindenfelser et al., 1979; Rajarathnam et al., 1979; Li and Eger-Hummel, 1979; Wicklow et al., 1980; Platt et al., 1981; Chang et al., 1981). Usually, the fungi fructify producing an excellent human food. At the same time, destruction of the lignocellulosic matrix takes place due to the intensive search for nitrogen by the fungi, which employ their enzymatic machinery very efficiently, one of the most potent found to date (Antheunisse, 1979, 1980, 1981). Dry matter biomass is lost by the action of the fungi. Cellulose and hemicellulose are hydrolyzed. Lignin is usually broken to smaller molecules which in principle can be further biodegraded. Under optimal conditions and with the proper fungi, the digestibility of the residue can increase by 100%.

A pretreated lignocellulosic material is more susceptible to biotransformation to ethanol or other chemicals. The many alternatives for biotransformation to date can be classified into two groups: indirect and direct processes. In the former, the basic objective is to hydrolyze the hollocellulose (residue that remains after lignin removal) to monomeric units employing chemicals, enzymes or both. Once this is done, the monomers are transformed into ethanol (Rolz et al., 1983). Glucose from cellulose is readily metabolized by brewer's and distiller's yeast. Hemicellulose hydrolysis forms mainly pentoses. To date there are no microorganisms available for the rapid conversion of pentoses to alcohol in sufficient quantities. This is a disadvantage of this type of process.

In the direct processes, the conversion of hollocellulose into ethanol is in one step avoiding the production and accumulation of monomers. The recent technical literature describes experiments where anaerobic bacteria have been able to convert hollocellulose to a spectrum of primary metabolites, among them ethanol (Su, 1978; Zeikus, 1979, 1980; Rosenberg, 1980; Wiegel and Ljungdahl, 1980; Flickinger, 1980; Averignos and Wang, 1980; Villet, 1981). Present research deals with the regulation and control of product distribution, genetic manipulations and selection and strain stability. One of the main problems has been the lack of tolerance by the bacteria, especially *Clostridium thermocellum*, to the high concentration of the products (Herrero and Gomez, 1980, 1981).

Another alternative which is being studied by ICAITI scientists is the so called "intermediate-acid." The idea is to transform hollocellulose to volatile organic acids, mainly acetic acid, as occurs in the rumen. The acids will then be biotransformed to ethanol employing alternate biochemical pathways. Two industrial companies in the USA have recently studied the acidification stage; they are Dynatech (Sanderson et al., 1978; Levy et al., 1981) and Exxon Research (Datta, 1981a, b). The acidification takes place under anaerobic and non-sterile conditions. The rate is increased by alkaline pretreatments of the lignocellulosic substrate. The water-soluble compounds and hemicelluloses are converted by 74-80% and cellulose by 37%. Two important advantages are the non-sterility and the conversion of both hemicelluloses and celluloses to the same final product. Khan and collaborators at NRC, Ottawa (Khan et al., 1977, 1978, 1979, 1981; Pate et al., 1980; Saddler and Khan, 1981) have isolated a bacterium identified as *Acetivibrio cellulolyticus* which transforms cellulose to organic acids with good yields. In principle, these acids can be transformed to ethanol. Lactic acid can be oxidized to pyruvic acid catalyzed by lactic dehydrogenase and then by known biochemical pathways to ethanol. Corry (1978) believes that this reaction takes place during putrefaction of living tissue. However, lactate dehydrogenation with hydrogen as electron acceptor is thermodynamically unfavorable and could take place under very low hydrogen pressures and without ATP synthesis (Thauer et al., 1977). Conversion of acetate to ethanol is thermodynamically more favorable and possibly has acetyl coenzyme A as intermediate.

The first reaction in this chain of bioevents still is the hydrolysis of hollocellulose. The most studied anaerobic cellulolytic bacteria are the ones present in the rumen: *Ruminoccus albus, R. flavefaciens, Bacteroides succinogenes, Butyrivibrio fibrisolvens and Fusobacterium polysaccharolyticum*. The enzymatic complex of the first three has been recently studied (Halliwell and Bryant, 1963; Leatherwood, 1965; Walker, 1967; Krishnamurthi and Kitts, 1969; Smith et al., 1973; Francis et al., 1978; Gawthorne, 1979; Yu and Hungate, 1979; Pettipher and

Latham, 1979; Groleau and Forsberg, 1981; Forsberg et al., 1981). Other cellulolytic anaerobes have also been studied: *Acetivibrio cellulolyticus, Clostridium thermocellum* and *Cl. acetobutylicum* (Lee and Blackburn, 1975; Gordon et al., 1978; Shinmyo et al., 1979; Ait et al., 1979; Ng et al., 1979; Rahmatulla et al., 1979; Khan, 1980; Saddler and Khan, 1980; Garcia et al., 1980; Khan et al., 1981; Allcock and Woods, 1981; Ng and Zeikus, 1981; Johnson et al., 1982; Mackenzie and Bilou, 1982). All have a complete extra- and intra-cellular cellulase complex and also hemicellulases. The endo- and exo-glucanases are extracellular and include cello- and xilo-biohydrolases. Beta glucosidase usually is cell-wall associated. For these microorganisms, there is a strong interaction between bacteria and fiber. Several mechanisms have been found experimentally: (i) strong surface attachment of the bacteria to the fiber, (ii) existence of enzymes packed in vesicles or satellites detached from the cell, and (iii) polymeric gel that retains the enzymes between bacteria and fiber (Leatherwood, 1973; Costerton et al., 1974a, b; Akin et al., 1974; Dinsdale et al., 1978; Morris and van Gylswyk, 1980; Forsberg et al., 1981). Aeration is not required, and high levels of mixing should be avoided as excess mixing seems unfavorable for bacteria-fiber interactions.

In summary, the "acid-intermediate" alternative offers new elements for the conversion of hollocellulose to ethanol or other products through organic volatile acids. However, the ideas require further exploration in the laboratory. Also, the pretreatment must be optimized. Direct processes seem to offer high promise of future use.

REFERENCES

Acock, C.W., J.K. Ward, I.G. Rush and T.J. Klopfenstein. 1979. Wheat straw and sodium hydroxide treatment in beef cow rations. Science 49:354.

Ait, N., N. Creuzet and P. Forget. 1979. Partial purification of cellulase from *C. thermocellum*. J. Gen. Microbiol. 113:399.

Akin, D.E., D. Burdick and G.E. Michaels. 1974. Rumen bacterial relationship with plant tissue during degradation revealed by transmission electron microscopy. Appl. Microbiol. 27:1149.

Allcock, E.R., and D.R. Woods. 1981. CMC and cellobiase production by *Clostridium acetobutylicum* in an industrial fermentation medium. Appl. Environ. Microbiol. 41:539.

Antheunisse, J. 1979. Observations on the decomposition of coconut fibres. J. Gen. Appl. Microbiol. 25:273.

Antheunisse, J. 1980. Breakdown of coconut fibres by *Trametes versi-color* and *Stereum rugosum*. J. Gen. Appl. Microbiol. 26:167

Antheunisse, J. 1981. Decay of coconut fibres by soil microorganisms. J. Gen. Appl. Microbiol. 27:435.

April, G.C., M.M. Kamal, J.A. Reddy, G.H. Bowers and S.M. Hansen. 1979. Delignification with aqueous-organic solvents. TAPPI 62:83.

April G.C., R. Bharoocha, J. Sheng and S. Hansen. 1982. Prehydrolysis achieves higher organosolv delignification. TAPPI 65:41.

Averignos, G.C., and D.C. Wang. 1980. Direct microbiological conversion of cellulosics to ethanol. Ann. Reports Ferment. Proccesses 4:165.

Averignos, G.C., and D.I.C. Wang. 1983. Selective solvent delignification for fermentation enhancement. Biotechnol. Bioeng. 25:67.

Baumeister, M., and E. Edel. 1980. Anthanol Wasser Aufschluss. Das Papier 34 (10A) V9.

Ben-Ghedalia, D., and J. Miron. 1981. Effect of sodium hydroxide, ozone and sulfur dioxide on the composition and *in vitro* digestibility of wheat straw. J. Sci. Food Agric. 72:224.

Ben-Ghedalia, B., G. Shelef and J. Miron. 1980. Effect of ozone and ammonium hydroxide treatments on the composition and *in vitro* digestibility of cotton straw. J. Sci. Food Agric. 31:1337.

Berg, F., and M. Mietz. 1980. Okonomische wertung der futter werterhohung von chemisch behandeltem stroh. Arch. Tierenahrung 30:311.

Berger, L., T. Klopfenstein and R. Britton, 1979. Effect of sodium hydroxide on efficiency of rumen digestion. J. Anim. Sci. 49:1317.

Berger, L., T. Klopfenstein and R. Britton. 1980. Effect of sodium hydroxide treatment on rate of passage and rate of ruminal fibre digestion. J. Anim. Sci. 50:745.

Bowers, G.H., and G.C. April. 1977. Aqueous n-butanol delignification of southern yellow pine. TAPPI 60:102.

Cabello, A., J. Conde and M.A. Otero. 1981. Prediction of the degradability of surgarcane cellulosic residues by indirect methods. Biotechnol. Bioeng. 23:2737.

Capper, B.S., D.J. Morgan and W.H. Parr. 1973. Alkali treated roughages for feeding ruminants: a review. Trop. Science 19:73.

Corry, J.E.L. 1978. A review. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. J. Appl. Bacteriol. 44:1.

Chang, S.T. 1978. *Volvariella volvacea*, p. 573. In: The Biology and Cultivation of Edible Mushrooms. Eds. S.T. Chang and W.A. Hayes. Academic Press, New York.

Chang, S.T., O.W. Lau and K.Y. Cho. 1981. The cultivation and nutritional value of *Pleurotus sajor-caju*. Eur. J. Appl. Microbiol. Bacteriol. 12:58.

Chesson, A. 1981. Effects of sodium hydroxide on cereal straws in relation to the enhanced degradation of structural polysaccharides by rumen microorganisms. J. Sci. Food Agric. 32:745.

Church, D.C., and K.A. Champs. 1980. Digestibility of untreated and hydroxide-treated animal ryegrass straw. J. Anim. Sci. 51:20.

Costerton, J.W., J.M. Ingram and H.J. Cheng. 1974. Structure and functions of the cell envelope of gram negative bacteria. Bacteriol. Rev. 38:87.

Costerton, J.W., H.N. Damgaard, and H.J. Cheng. 1946. Cell envelope morphology of rumen bacteria. J. Bacteriol. 118:1132.

Datta, R. 1981a. Acidogenic fermentation of corn stover. Biotechnol. Bioeng. 23:61.

Datta, R. 1981b. Acidogenic fermentation of lignocellulose-acid yield and conservation of components. Biotechnol. Bioeng. 23:2167.

Dekker, R.F.H., and G.N. Richards. 1972. Effects of sulphate ion on *in vitro* rumen digestion of polysaccharide constituents of bagasse. Aust. J. Biol. Sci. 25:1377.

Deschamps, A.M., J.P. Gillie and J.M. Lebeault. 1981. Direct delignification of untreated bark chips with mixed cultures of bacteria. Eur. J. Appl. Microbiol. Biotechnol. 13:225.

Dinsdale, D., E.J. Morris and J.S. Bacon. 1978. Electron microscopy of the microbial populations present and their modes of attack on various cellulosic substrates undergoing digestion in sheep rumen. Appl. Environ. Microbiol. 36:160.

Dobie, J.B., and H.G. Walker. 1977. Effects of sodium hydroxide and ammonia on cubability and digestibility of rice straw. Trans. Am. Soc. Agr. Eng. 20:1016.

Eger, G. 1978. Biology and breeding of *Pleurotus*, p. 497. In: The Biology and Cultivation of Edible Mushrooms. Eds. S.T. Chang and W.A. Hayes. Academic Press, New York.

Eger, G., S.F. Li and H. Leal. 1979. Contribution to the discussion on the species concept in the *Pleurotus ostreatus* complex. Mycol. 71:577.

Flickinger, M.D. 1980. Current biological research conversion of cellulosic carbohydrates into liquid fuels. Biotechnol. Bioeng. 22, Suppl. 1:27.

Forsberg, C.W., T.J. Beveridge and A. Hellstrom. 1981. Cellulase and xylanase release from *Bacteroides succinogenes* and its importance in the rumen environment. Appl. Environ. Microbiol. 42:886.

Francis, G.L., J.M. Gawthorne and G.B. Stover. 1978. Factors affecting the activity of cellulases isolated from the rumen digesta of sheep. Appl. Environ. Microbiol. 36:643.

Garcia, D.V., A. Shinmyo, A. Madia and A.L. Demain. 1980. Studies on cellulase production by *Clostridium thermocellum*. Eur. J. Appl. Microbiol. Biotechnol. 9:189.

Garrett, W.N., H.G. Walker, G.O. Kobber and M.R. Hart. 1979. Response of ruminant to diets containing sodium hydroxide or ammonium treated rice straw. J. Anim. Sci. 48:92.

Gawthorne, J.M. 1979. Extracellular carbohydrase complex from rumen contents. An. Rech. Vet. 10:249.

Gordon, J., M. Jimenez, C.L. Cooney and D.I.C. Wang. 1978. Sugar accumulation during enzyme hydrolysis and fermentation of cellulose. Am. Inst. Chem. Eng. Symp. 74:91.

Green, J., and N. Sawyer. 1982. Alkaline pulping in aqueous alcohols and amines. TAPPI 65:133.

Groteau, D., and C.W. Forsberg. 1981. Cellulolytic activity of the rumen bacteria *Bacteroides succinogenes*. Can. J. Microbiol. 27:517.

Halliwell, G., and M.P. Bryant. 1963. The cellulolytic activity of pure strains of bacteria from the rumen of cattle. J. Gen. Microbiol. 32:441.

Han, Y.W., E.A. Catalano and A. Ciegler. 1983. Chemical and physical properties of sugarcane bagasse irradiated with gamma rays. J. Agric. Food Chem. 31:34.

Hansen, S.M., and G.C. April. 1982. Prediction of solvent effects in aqueous-organic solvent delignification. Ind. Eng. Chem. Prod. Res. Dev. 21:621.

Herrero, A.A., and R.F. Gomez. 1980. Development of ethanol tolerance in *Clostridium thermocellum:* effect of growth temperature. Appl. Environ. Microbiol. 40:472.

Herrero, A.A., and R.F. Gomez. 1981. Inhibition of *Clostridium thermocellum*, vol. II, p. 213. In: Advances in Biotechnology. Eds. M. Moo-Young and C.W. Robinson. Pergamon Press, Toronto.

Higgins, A.J. 1981. The effect of a sodium hydroxide spray treatment on digestibility of barley straw in sheep and goats. Agr. Wastes 3:145.

Ibrahim, M.N.M., and G.R. Pearce. 1983. Effects of gamma radiation on the composition and *in vitro* digestibility of crop byproducts. Agr. Wastes 2:253.

Ibrahim, M.N.M., and G.R. Pearce. 1983. Effects of chemical pretreatments on the composition and *in vitro* digestibility of crop byproducts. Agr. Wastes 5:135.

INIP. 1983. Comunicacion personal (FB Irma Tejada de Hernandez, Jefe, Departmamento de Analisis instrumental, Institutio Nacional de Investigaciones Pecuarias, Palo Alto, D.F., Mexico.

Jackson, M.G. 1977. The alkali treatment of straw. Anim. Feed Sci. Technol. 2:105.

Janshekar, H., and A. Fiechter. 1982. On the bacterial degradation of lignin. Eur. J. App. Microbiol. Biotechnol. 14:47.

Jayasuriya, M.C.N., and H.G.D. Perera. 1982. Urea-ammonia treatment of rice straw to improve its nutritive value for ruminants. Agr. Wastes 4:143.

Johnson, E.A., M. Sakajoh, G. Halliwell, A. Madia and A.L. Demain. 1982. Saccharification of complex cellulose substrates by the cellulase system from *Clostridium termocellum*. Appl. Env. Microbiol. 43:1125.

Johnson, E.A., M. Sakajoh, G. Halliwell, A. Madia and A.L. Demain. 1982. Saccharification of complex cellulose substrates by the cellulase system from *Clostridium termocellum*. Appl. Env. Microbiol. 43:1125.

Katzen, R., R. Frederickson and B.F. Brush. 1980. The alcohol pulping and recover process. Chem. Eng. Prog. 76 (2) 62.

Khan, A.W. 1977. Anaerobic degradation of cellulose by mixed cultures. Can J. Microbiol. 23:1700.

Khan, A.W., and T.M. Trottier. 1978. Effect of sulfar-containing compounds on anaerobic degradation of cellulose to methan by mixed cultures obtained from the sewage sludge. Appl. Environ. Microbiol. 35:1027.

Khan, A.W., T.M Trottier, G.B. Patel and S.M. Martin. 1979. Nutrient requirement for the degradation of cellulose to methane by a mixed population of anaerobes. J. Gen. Microbiol. 112:365.

Khan, A.W. 1980. Degradation of cellulose to methane by a coculture of *Acetivibrio cellulolyticus* and *Methanosarcina barkeri*. FEMS Microbiol. Letters 9:233.

Khan, A.W., D. Wall and L. van den Berg. 1981. Fermentative conversion of cellulose to acetic acid and celluloytic enzyme production by a bacterial mixed culture obtained from sewage sludge. Appl. Environ. Microbiol. 41:1214.

Kleinert, T. 1975. Ethanol-water delignification of wood-rate contents and activation energy. TAPPI 58:170.

Kleinert, T. 1974. Organosolv pulping with aqueous alcohol. TAPPI 57:99.

Krishnamurti, C.R., and W.D. Kitts. 1969. Preparation and properties of cellulases from rumen microorganisms. Can. J. Microbiol. 15:1373.

Leatherwood, J.M. 1965. Cellulase from *R. albus* and mixed rumen microorganisms. Appl. Microbiol. 13:771.

Lee, B.H., and T.H. Blackburn. 1975. Cellulase production by a thermo philic *Clostridium* species. Appl. Microbiol. 30:346.

Lesoing, G., T. Klopfenstein, I. Rush and J. Ward. 1980. Chemical treatment of wheat straw. J. Anim. Sci. 51:263.

Levy, P.F., J.E. Sanderson, R.G. Kispert and D.L. Wise. 1981. Biorefining of biomass to liquid fuels and organic chemicals. Enzyme Microbiol. Technol. 3:207.

Li, S.F., and G. Eger-Hummel. 1981. Genetic control of dikaryotic fruiting in the basidiomycete *Pleurotus ostreatus*, Vol. I, p. 101. In: Advances in Biotechnology. Eds. M. Moo-Young, C.W. Robinson and C. Vezina Robinson. Pergamon Press, Toronto.

Lindenfelser, L.A., R.W. Detroy, I.M. Ramstak and K.A. Worden. 1979. Biological modification of the lignin and cellulose components of wheat straw by *Pleurotus ostreatus*. Dev. Ind. Microbiol. 20:541.

McKenzie, C.R., and D. Bilous. 1982. Location and kinetic properties of the cellulase system of *Acetivibrio celluloyticus*. Can J. Microbiol. 28:1158.

Martin, P.C., T.C. Cribeiro, A. Cabello and A. Elias. 1974. The effect of sodium hydroxide and pressure on the dry matter digestibility of bagasse and bagasse pith. Cuban J. Agric. Science 8:21.

Morton, R., and S. Granzow. 1982. Ethanol-alkali pulping. TAPPI 65:103.

Morris, E.J., and N.D. van Gylswyk. 1980. Comparison of the action of rumen bacteria on cell walls from *Eragrotistef*. J. Agric. Sci. 95:313.

Neilson, M.J., F. Shafizadeh, S. Aziz and K.W. Sarhanen. 1983. Evaluation of organosolv pulp as a suitable substrate for rapid enzymatic hydrolysis. Biotechnol. Bioeng. 25:609.

Ng, T.K., P.J. Weimer and J.G. Zeikus. 1977. Cellulolytic and physiological properties of *Clostridium thermocellum*. Arch. Microbiol. 114:1.

Ng, T.K., and J.G. Zeikus. 1981. Comparison of extracellular cellulose activities of *Clostridium thermocellum* LQRI and *Trichoderma resei* QM 9414. Appl. Env. Microbiol. 42:231.

Odier, E., G. Janin and B. Monties. 1981. Poplar lignin decomposition by gram-aerobic bacteria. Appl. Environ. Microbiol. 41:337.

Patel, G.B., A.W. Kahn, B.J. Agnew and J.R. Calvin. 1980. Isolation and characterization of an anaerobic, cellulolytic microorganism, *Acetivibrio cellulolyticus* gen nov sp nov. Int. J. Syst. Bacteriol. 30:179.

Pettipher, G.L., and M.J. Latham. 1979. Characteristics of enzymes produced by *Ruminoccus flavefacines* which degrade plant cell walls. J. Gen Microbiol. 110:21-29.

Plasse, F., M.D. Fardeau and J.P. Belaich. 1980. Microcolorimetric evaluation of the efficacy of the alkaline treatment on the fermentation of straw. Bacteriol. Lett. 2:11.

Platt, M.W., I. Chet and Y. Henis. 1981. Lignocellulose degradation during growth of the mushroom *Pleurotus* sp "Florida" on cotton straw. Eur. J. Appl. Microbiol. Biotechnol. 13:194.

Preston, R.R. 1975. Sugarcane as the basis for intensive animal production in the tropics. Proc. Conf. on Anim. Feeds of Tropical and Subtropical Origin, TPI, London, p. 69.

Rahmatullah, M., A. Rahmau, A.A. Chowdburg and M.A. Rashid. 1979. Anaerobic digestion of jute-stick powder by a mesophilic celluloytic *Clostridium* species. J: Ferment. Technol. 57:117.

Rajarathnam, S., D.B. Wankhede and M.V. Patwardhan. 1979. Some chemical and biochemical changes in straw constituents during growth of *Pleurotus flabellatus* (Berk and Br) Sacc. Eur. J. Appl. Microbiol. 8:125.

Randel, P.F. 1972. A comparison of the digestibility of two complete rations containing either raw or alkali-treated sugarcane bagasse. J. Agr. Univer. Puerto Rico 56:18.

Rexen, F. 1979. Low quality forages improve with alkali treatment. Feedstuffs 51 (42) 33.

Rolz, C., S. de Cabrera, F. Calzada, R. Garcia, R. de Leon, M.C. de Arriola, F. de Micheo and E. Morales. 1983. Concepts in the biotransformation of carbohydrates to ethanol. Adv. Biotechnol. Proc. 1:97.

Rosenberg, S.L. 1980. Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. Enzyme Microb. Technol. 2:185.

Saddler, J.N., and A.W. Khan. 1980. Cellulose Production by *Acetivivibrio cellulolyticus*. Can. J. Microbiol. 26:760.

Saddler, J.N., and A.W. Kahn. 1981. Cellulolytic enzyme system of *Acetivibrio cellulolyticus*. Can. J. Microbiol. 27:258.

Sanderson, J.E., D.L. Wise and D.C. Augenstein. 1978. Organic chemicals and liquid fuels from algal biomass. Biotechnol. Bioeng. Symp. 8:131.

Sarhanen, K.V. 1980. Acid catalyzed delignification of lignocellulosics in organic solvents. Prog. Biomass Conv. 2:128.

Shinmyo, A., D.V. Garcia and A.L. Demain. 1979. Studies on the extracellular cellulolytic enzyme complex by *Clostridium thermocellum*. J. Appl. Biochem. 1:202.

Singh, N.S., and S. Rajarathnam. 1979. *Pleurotus eous* (Berk) Sacc.: a new cultivated mushroom. Current Science 46:617.

Smith, W.R., I. Yu and R.L. Hungate. 1973. Factors affecting cellulolysis by *R. albus*. J. Bacteriol. 114:729.

Solaiman, S.G., G.W. Horn and F.N. Owens. Ammonium hydroxide treatment of wheat straw. J. Anim. Sci. 49:802.

Su, T.M. 1978. Bioconversion of plant biomass to ethanol. Am. Inst. Chem. Eng. Symp. Series 74:75.

Thauer, R.K., K. Jungerman and K. Deker. 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Reviews 41:100.

van Soest, P.J., and J.B. Robertson. 1980. Systems of analysis for evaluating fibrous feeds. In: Standardization of Analytical Methodology for Feeds. Eds. W.J. Pidgen, C.C. Balch and M. Graham. International Development Research Centre, IRDR-134e, Ottawa.

Villet, R. 1981. Biotechnological research and development for biomass conversion to chemical and fuels. Deve. Ind. Microbiol. 22:97.

Walker, D.J. 1967. Some properties of xylanase and xelobiose from mixed rumen microorganisms. Aust. J. Biol. Sciences 20:799.

Wicklow, D.T., R.W. Detroy and B.A. Jessee. 1980. Decomposition of lignocellulose by *Cyathus stercoreus* (Sch) de Toni NRRL 6473, a white rot fungus from cattle dung. Appl. Environ. Microbiol. 40:169.

Wiegel, J., and L.G. Ljungdahl. 1980. Ethanol as fermentation product of extreme thermophilic anaerobic bacteria. 4th Symp. Techn. Microbiol. (Berlin), 117.

Wilkinson, J.M., and R. Gonzalez. 1978. Ensiled alkali treated straw. I. Effect of level and type of alkali on the composition and digestibility *in vitro* of ensiled barley straw. Anim. Feed Science Technol. 3:117.

Willis, C.M., O.T. Stallup and D.L. Kreider. 1980. Influence of sodium hydroxide and enzyme additions on nutritive value of rice straw. J. Anim. Sci. 50:303.

Yu, I., and R.E. Hungate. 1979. The extracellular cellulases of *R. albus*. Ann Rech. Vet. 10:251.

Zadrazil, F. 1977. The conversion of straw into feed by basidiomycetes. Eur. J. Appl. Microbiol. 4:273.

Zadrazil, F. 1978. Cultivation of *Pleurotus*, p. 521. In: The Biology and Cultivation of Edible Mushrooms. Eds. S.T. Chang and W.A. Hayes. Academic Press, New York.

Zadrazil, F. 1979. Screening of basidimoycetes for optimal utilization of straw, p. 139 In: Straw Decay and its Effect on Disposal and Utilization. Ed. E. Grossbard. John Wiley & Sons, Chichester.

Zadrazil, F. 1980a. Influence of ammonium nitrate and organic supplements on the yield of *Pleurotus sajor caju* (Fr) Sing. Eur. J. Appl. Microbiol. Biotechnol. 9:31.

Zadrazil, F. 1980b. The influence of ammonium nitrate supplementation on degradation and *in vitro* digestibility of straw colonized by higher fungi. Eur. J. Appl. Microbiol. Biotechnol. 9:37.

Zadrazil, F. 1980c. Conversion of different plant waste into feed by *Basidiomycetes* 9:243.

Zadrazil, F. 1980d. Factors determining lignin decomposition and *in vitro* digestibility of lignocellulose during solid state fermentation, Vol II, p. 369. Eds. M. Moo-Young and C.W. Robinson. Pergamon Press, Toronto.

Zeikus, J.G. 1979. Thermophilic bacteria: ecology, physiology and technology. Enzyme Microb. Technol. 1:243.

Zeikus, J.G. 1980. Chemical and fuel production by anaerobic bacteria. Ann. Rev. Microbiol. 34:423.

ROUND TABLE ON BIOTECHNOLOGY PROCESS

Victor Yang

Senior Engineer
PROMON Engineering, S.A.
Sao Paulo, SP, Brazil

Rodolfo Quintero R.

Director, Departamento de Tecnologia de Alimentos
Instituto de Investigaciones Biomedica
Universidad Nacional de Mexico
Apartado 70228
0510, México, D.F., México

Gustavo Viniegra G.

Chief of Microbiology
Universidad Autonoma Metropolitan-Iztapalapa
Iztapalapa, México 13

The primary objective of these discussions was to identify the key elements of a viable strategy to implement a biotechnology program in the Americas, emphasizing developing countries which may benefit from proper usage of biotechnology. The scope was limited to industrial biotechnology processes.

SCOPE OF BIOTECHNOLOGY IN THE AMERICAS

There are significant activities in biotechnology at the industrial level in many of the countries in the Americas. Operating plants producing ethanol as fuel and chemical feedstock; food and feed additives such as lactic acid, single-cell protein, citric acid, monosodium glutamate and lysine; and pharmaceuticals such as penicillin illustrate the current state of the biotechnology industry in the region. The potential of biotechnology to contribute effectively toward the industrial and socio-economic development of the Americas hinges upon proper planning and optimal utilization of the local mix of resources and market. Every effort should be made to identify and to exploit local advantages in resources, personnel and market.

The ability of biotechnology to convert widely available resources such as sunshine and biomass to products that meet local needs suggests the important developmental role that it may play. This potentially important role should be conveyed to the government officials, to the business community and to society at large in the Americas.

PLANNING AND ANALYSIS

Judicious selection of biotechnology processes which will ultimately provide net positive socio-economic returns from investments is complicated both by the large number of products and processes that may in theory be considered and by the different set of circumstances and the hierarchy of needs throughout the Americas. Common constraints in most developing countries in the region include shortages of capital and hard currencies and of skilled labor, properly trained for the requirements of the emerging biotechnology industry.

Planning for biotechnology industries should consider specific socio-economic parameters and local priorities. In some countries, food and food production are limiting, whereas in other countries, the supply of energy is of prime interest. Examples of current and potential applications of biotechnology that merit attention are in Table 1.

Table 1. Examples of current and potential application of biotechnology in the Americas

NEAR TERM	*INTERMEDIATE TO LONG TERM*
Ethanol fermentation	Penicillins
Single-cell protein from biomass	Human and animal vaccines
Biogas	Production of hormones by genetically engineered organisms
Aminoacids (lycine and threonine)	Bioprocesses for mining industry
Bagasse digestion	Biomaterials for petroleum exploration
Nitrogen-fixing bacteria	Food additives and biomaterials for waste treatment
Organic acids	

TRANSITION FROM THE TRADITIONAL TO THE NEW BIOTECHNOLOGY

The current biotechnology industry in the region is based mainly on traditional biotechnology such as alcohol fermentation. New biotechnology including modern techniques such as those pertaining to genetic engineering offers the potential for lowering costs and widening the scope of the industry. The realization of such potential requires proper management of the transition from the traditional. Traditional industries such as feedstuffs and alcohol fermentation should, however, receive continued and additional attention as they account for a large number of jobs and contribute significantly to the economy in the rural areas. The economic and human costs of modernizing or revamping these industries should be considered in the planning for the introduction of new biotechnology.

LINKAGE BETWEEN RESEARCH AND MARKET PLACE

There was concern that much of biotechnology research may not ultimately benefit the consumer and the society at large. Effective research and implementation strategy should provide the following factors:

1. Communication: Both lateral communication among research institutions and vertical communication between research institutions, industry and government should be enhanced to avoid duplication or misdirection.
2. Institutional linkage form: Private industry should engage in on-going biotechnology-related activities to promote rapid transfer of research results to the market place. Multilateral non-governmental institutions such as Interciencia and the Interamerican Development Bank may help form the important linkages.
3. Economics: Micro-economic feasibility as well as social considerations should be major driving forces in defining research that is relevant to the market place.

IMPACT ON AGRICULTURE AND INDUSTRY

Comprehensive technology assessment is required to maximize contributions and to minimize negative impact on existing agricultural and industrial activities. Brazil's experience illustrates the major effects of a sizable commitment of resources to convert biomass to liquid fuels on traditional agriculture and on automotive and refinery industries. The creation of new jobs and the savings on imported petroleum should be weighed against the costs of modifying refinery operations, a changing energy consumption pattern and other country-wide shifts.

INTERNATIONAL COOPERATION

The multidisciplinary nature and wide span of biotechnology processes underscore the importance of international cooperation. Very few countries have themselves the combined requirements of critical mass of trained personnel, capital, technology, adequate market size and sufficient raw materials that are needed to sustain a viable biotechnology industry. Cooperation among nations should be promoted at both the research and the applied levels.

In research, joint programs addressing common problems may enable smaller countries to participate in important biotechnological developments. People-to-people contact should be encouraged to accelerate exchange of information.

At the applied level, joint ventures between research institutions and industry should be considered in order to integrate technological innovations to commercial-scale production facilities. Joint ventures ought to involve parties from different countries if complementary strengths are identified.

Barriers to effective international cooperation such as legislation concerning intellectual property, e.g., patents, and trade of materials related to biotechnology industry should be discussed by the countries involved. The role of non-government agencies, e.g., Interciencia, in fostering international cooperation both at the research and applied levels should be explored and exploited.

ROUND TABLE ON APPLIED GENETIC ENGINEERING

Pedro Leon

Centro de Biología Molecular y Cellular
Universidad de Costa Rica,
San Jose, Costa Rica

Juan Dellacha

Consejo de Investigaciones de la Provincia de Buenos Aires
Buenos Aires, Argentina

Jorge Allende

Departamento de Bioquimica
Universidad de Chile
Santiago, Chile

The discussions centered on four main issues:

1. SHOULD BIOTECHNOLOGY IN LATIN AMERICA BE DEVELOPED THROUGH THE ESTABLISHMENT OF A SINGLE REGIONAL BIOTECHNOLOGY CENTER?

Consensus was to emphasize the use of limited funds to support national research and technology groups and centers instead of backing a large, single regional center. It was agreed, however, that there should be a mechanism to coordinate the efforts and to stimulate collaboration among the research and technology groups of the region. The formation of an organized network of Latin American biotechnology laboratories was suggested. Latin America should also participate in the proposed UNIDO International Center for Biotechnology and Genetic Engineering. The Latin America governments should insist that significant financial and scientific support from this center be available to the proposed Latin American network.

2. SHOULD ALL LATIN AMERICAN BIOTECHNOLOGY EFFORTS CONCENTRATE ON ATTACKING A SINGLE PROBLEM?

Consensus was that the different conditions, resources and priorities of the Latin American countries would make it very difficult to concentrate all efforts in a single project. It was agreed, however, that there are many pressing common problems that involve most of the Latin American countries. High priority should be given to identifying those problems and choosing effective national and regional strategies to attack them.

3. WHAT COLLABORATIVE ACTIVITIES SHOULD BE UNDERTAKEN TO STRENGTHEN BIOTECHNOLOGY IN THE LATIN AMERICAN REGION?

The most important area for collaboration is the development of human resources for the different areas of biotechnology. In this respect, short training courses and long-term fellowships are needed within the region. Another important area for collaboration is information. Modern computer facilities are needed to help store and organize information. Also, an "information clearing-house" should be established to receive and distribute biotechnological information. A newsletter, a widely distributed journal or both are needed. INTERCIENCIA could have an important role.

Another useful collaborative project is a regional directory of laboratories, plants, individuals, training programs and other resources for biotechnology.

Regional collaboration could develop a system to assign under small contract the preparation and distribution of specific reagents such as restriction enzymes, radioactive nucleotides and monoclonal antibodies. These contracts would go to laboratories within the region. Distribution of the reagents would be to those within the region who need them.

Collaboration between Latin American laboratories could be facilitated by travel funds for exchange of scientists.

4. WHAT SHOULD LATIN AMERICAN GOVERNMENTS DO TO PROMOTE THE INTEGRATION OF THE ADVANCES OF THE BASIC BIOTECHNOLOGY SCIENCES WITH THEIR POSSIBLE APPLICATIONS?

Governments may loan funds to support research by industries. This is now being done by Spain and Mexico. Tax incentives and industrial grants for research as in the USA and other developed countries are also of potential value and should be examined.

Scientists should take every opportunity to communicate to governments the importance of the applications of biotechnology.

Each participant of the symposium should make an effort upon return to his country to define and promote biotechnology in terms of areas, problems and priorities appropriate for that country and to the region.

ROUND TABLE ON BIOTECHNOLOGY IN AGRICULTURE — IMPORTANCE FOR DEVELOPING COUNTRIES

Maro Ran-ir Sondahl

Department of Genetics
Instituto Agronómico de Campinas
Caixa Postal 28
13100 Sao Paulo, SP, Brazil

Oscar Arias Morera

Centro de Biologia Molecular y Celular
Universidad de Costa Rica
San José, Costa Rica

J.R. Jardim Freire

Professor, Departamento do Solos
Faculdade De Agronomica
Universidade Federal do Rio Grande do Sul
Caixa Postal 776, 90.000 Porto Alegre, R.S., Brazil

Luding Muller

Centro Agronómico Tropical de Investigación Ensenanza
Turrialba, Costa Rica

Otto Crocomo

Coordenador, Centro de Biotecnologia Agricola
Fundacao de Estudios Agrarios Luiz de Queiros
Caixa Postal 9
13400 Piracicaba, SP, Brazil

Glenn Collins

Professor, Department of Agronomy
University of Kentucky, N-122 Agricultural Science Building North
Lexington, Kentucky 40506 U.S.A.

VEGETATIVE PROPAGATION

Consensus held that immediate- medium- and long-term benefits to the efficient improvement of tropical crops could be obtained by utilizing tissue and cell culture in conjunction with plant breeding and other conventional crop improvement techniques. The utilization of specific methods depends on (i) the problems posed for individual crops in each country, and (ii) the availability of trained personnel, facilities and support for the research. Methods available for short-term application include meristem and embryo culture. Application in the medium term include the production of haploids from anther culture and the selection of useful somaclonal mutants from cell cultures. Long-term applications of cells in culture include the production of secondary products and genetic engineering of genes into plants.

Vegetative propagation offers both short-term return and enormous potential for developing countries. This technique seems very attractive for tropical species that have a high degree of heterozygosity, suffer virus infection and are mainly propagated by vegetative means. Examples where vegetative propagation is important include palms (oil palm, peach palm), agave, bamboo, orchids, coffee, banana, plantain, calocasia, cassava, potato and pineapple. Vegetative propagation can be adopted by germplasm banks to facilitate the availabilty and exchange of important tropical plant species as well as to preserve some endangered native plants.

Attempts to apply vegetative propagation in developing countries fall short both from the lack of trained personnel and from the lack of associations of private and government institutions to accomplish "scale up" and delivery to farmers. Private enterprises are moving in applying *in vitro* vegetative propagation techniques for a few tropical species like oil palm (Costa Rica, Dominican Republic), banana (Honduras), orchids (Brazil) and some ornamentals like carnation, crysanthemum and bromelia (Costa Rica, Colombia). Government efforts in some Latin American countries are applying *in vitro* techniques for virus elimination and vegetative propagation of oil palm (Costa Rica, Brazil, Dominican Republic, Colombia), peach palm (Costa Rica), strawberry (Brazil), potato (Brazil), citrus (Brazil), and coffee (Costa Rica, Mexico).

SECONDARY PRODUCTS

Only a few Latin American countries are now using techniques of plant cell suspension cultures, e.g., Brazil, Argentina and Mexico. The development of human resources for biotechnology, especially in the field of plant cell and tissue culture, is **urgent.** Competence in plant genetics, plant biochemistry, plant physiology and correlated disciplines is also needed.

For most Latin American countries, the production of secondary metabolites through plant cell suspension cultures in bioreactors is a long- or, at least, a medium-term, prospect. The application of this technique may be easier to implement in laboratories where plant cell and tissue culture are already established. The approach of using bioreactors for secondary products should not preclude using somaclonal variation to create new plant variants and screening for production of natural products.

The cloning of plant genes in bacteria has the potential to increase the efficiency of production of secondary products. Biotransformation of from one to three enzymes would be feasible. Presently, plant genes are poorly understood and difficult to isolate.

Bioengineering and plant-cell teams should cooperate in joint projects to scale up the production of secondary products in plant-cell fermenters.

NITROGEN FIXATION

The potential savings in fossil-derived nitrogen through biological nitrogen fixation is of prime importance to the developing countries in Latin America. The region as a whole is a net importer of nitrogen fertilizers; this requires hard currencies. The case of the Brazilian soybean industry is noteworthy as soybeans are grown on about 12 million hectares. Since legumes are an excellent source of protein, substantial effort should be devoted to make them more available. Attention should also be given to local leguminous plants of economic and social importance such as native forages and field beans (*Phaseolus vulgaris*).

Inoculant production should be initially stimulated by local governments for use in demonstration plots, for farm distribution and to function as standards for future private inoculant businesses. Assistance should be sought from international organizations such as FAO, UNESCO, BID, etc. Control of inoculant is critical to avoid damage to farmers and to governmental programs. Application of modern biotechnology techniques is recommended to improve local strains and to create more efficient nitrogen-fixing systems. Again, training of scientists is needed.

Table 1 lists suggested priorities for biological nitrogen fixation (BNF) for developing countries. Final priorities must be established in each country or area for specific legumes and in consideration of availability of resources.

GENETICS

There is enormous potential in the use of *in vitro* techniques to generate new sources of variability for local breeding programs. Agriculture is based on varieties adapted to specific ecological niches. Breeding should provide genotypes adequate to the various local needs.

The applications of *in vitro* techniques to genetics and plant breeding include: induced mutation and selection, organelle transfer, genetic transformation, somatic hybridization, haploid production, embryo culture and somaclonal variation.

The necessity of using highly regenerating cell systems for induced mutation was stressed. This technique requires a long time to apply to crop improvement. Random and diverse modification of the plant genome, e.g., deletions, can adversely affect important agronomic characteristics.

*Table 1. Suggested priorities in biological nitrogen fixation (BNF) for developing countries**

ACTIVITIES	EARLY ADVANCE STAGE	MEDIUM STAGE	ADVANCE STAGE
A. Rhizobium/Legumes			
1. Strains local selection			
a. Efficiency in fixation	+ + +	+ + +	+ + +
b. Competitiveness, survival in soil and peat, stress tolerance and soil N	+	+ +	+ + +
2. Technology of inoculation	+	+ +	+ + +
3. Technology of inoculant production	+	+ +	+ + +
4. Effect of pesticides	+	+	+ + +
5. Limiting factors-abiotic and biotic	+ + +	+ + +	+ + +
6. Inoculant production	+ + +	+ + +	+ + +
7. Inoculant quality control	+ + +	+ + +	+ + +
8. Extension/research integration	+ + +	+ + +	+ + +
9. Development of better plant genotypes	+	+ +	+ + +
10. Improvement of BNF by genetic means	+	+	+ + +
B. Other BNF systems	+	+	+ + +

* Priorities must be established by country and/or area, specific legumes, and availability of resources.

\+ Low priority + + Medium priority + + + High priority.

To date, the transfer of organelles has presented many difficulties including isolation, survival in the foreign cytoplasm and expression of the organelle traits. Male sterility, organelle recombination and herbicide tolerance are important areas to explore.

Transformation of plant cells using molecular genetic techniques for genome modification and crop improvement is a long term research area of the highest priority. *In vitro* cellular systems must be established in order to utilize molecular genetics in crop improvement.

At present, a great deal of effort is directed to develop efficient vectors such as the Ti plasmid, viruses and liposomes for DNA insertion into plant protoplasts. Some potential applications in developing countries include modified invertase in sugarcane, cyanide in cassava, hemaglutinin in soybean, and oxidizing-enzyme systems in oil-palm fruits.

Somatic hybridization was recognized as another powerful tool for the generation of novel plant types which may be agronomically important in tropical regions. The application of this technique was considered a medium-term approach for crop improvement.

Haploid culture of F_1 hybrids can speed up breeding programs and facilitate genetic analysis and selection at the haploid stage. This technique was recognized as a middle-term approach.

The use of embryo rescue to recover F_1 and BC_1 interspecific hybrids was mentioned as an immediate (short-term) application of an *in vitro* technique for crop improvement. The simplicity and the wide application of this technique make it very attractive to any Latin American country.

Finally, somaclonal variation (or protoclonal variation) was recognized as a technique of great potential for crop improvement if followed by proper selection and coupled with expertise from an experienced team of plant breeders. This technique may lead to short-term release of varieties if one explores at a maximum the natural variability of somatic cells from adapted varieties. There is, for example, a possibility of selecting agave with a shorter life cycle, better disease resistance and improved qualitative and quantitive characteristics.

ROUND TABLE ON HUMAN AND ANIMAL HEALTH

Jorge Elias Kalil

Centro de Biotecnologia
Porto Alegre, Brazil

Carlos Rolz

Head, Applied Research Division
ICAITI
Guatemala City, Guatemala

Cristian Orrego

Visiting Associate
National Institutes of Health
Bethesda, Maryland 20205 U.S.A.

Transmissible diseases remain the dominant cause of human morbidity and mortality in many countries. In some areas of the world, the ominous cycle of malnutrition and infection results in the death of up to 40% of children before five years of age. Besides adequate human nutrition, immunization is one of the most effective means of preventing infectious diseases provided suitable public-health programs can be implemented.

Developments in the biomedical sciences during the last twenty years offer the prospect of not only improving available vaccines but also for the production of new vaccines against many infectious agents and parasites which, until now, have been refractory to this approach. This new ability raises the question of what should be the priorities in human and animal disease research for design of new vaccines. The assessment of the funding priorities for the development of vaccines that was made at the workshop on Priorities in Biotechnology Research for International Development sponsored by the U.S. National Research Council* is a useful document for decision making in this area. However, it was hard to define one consistent set of guidelines applicable to all Latin American nations.

Research on local or regional diseases that are not receiving attention elsewhere should be undertaken. Hemorrhagic fever in Argentina and Chagas disease in Brazil are examples. The development of animal vaccines against brucellosis and bovine leukosis, of obvious economic importance to many countries in Latin America, will most likely also receive priority. These diseases are not being currently studied with the methods of modern biology. On the other hand, even though hepatitis B is being intensively studied in the U.S. and Europe, it could be justified for Brazil to consider assigning some resources to research in this area as it already has the capability to produce sophisticated diagnostic reagents for detecting this disease.

Developed countries have sometimes funded investigations in their own countries on diseases prevalent in other nations. This has, in turn, resulted in collaboration with research groups in countries subject to the particular diseases. Decisions on priorities should include this possibility along with other factors.

The second question that concerned this roundtable was the access to vaccines. Large segments of the population in many countries can not afford them through the open market. The recognition of health as a human right has led national governments to take the responsibility of implementing programs of mass immunization reaching individuals regardless of their income. This will also have to be the case with future vaccines. State vaccine institutes in many countries are already producing safe products for public immunization. Bilateral as well as multilateral arrangements involving appropriate international agencies and governments might be required in the future. Joint ventures between, for example, a state-supported company and a private biotechnology concern could conceivably be another avenue for mass production of vaccines. Perhaps, an International Biotechnology Institute for Vaccine Production could be a future source of vaccines for particular regions of the world. It is clear that new and imaginative schemes are necessary as there is little indication that pharmaceutical houses from the private

**Priorities in Biotechnology: Research for International Development. Proceedings of a workshop, Washington, D.C. and Berkeley Springs, West Virginia, July 26-30, 1982. National Academy Press, Washington, D.C., 1982.*

sector will, on their own, develop and produce vaccines against, for example, tropical parasites of humans. Yet, the tools of biotechnology that are now available make it possible for the first time to conceive of strategies for the development of vaccines against some of the major tropical parasites.

Animal health has to be seen not only as a domain of disease control but also of animal nutrition. Biotechnology offers the opportunity to expand the utilization of both traditional and underexploited raw materials for a range of products and processes important to animal nutrition. A variety of strategies appear possible. Materials high in cellulosic biomass such as sugarcane bagasse, lemon grass bagasse, sawdust, forestry residues, and aquatic plants as well as certain industrial effluents can now be considered as starting materials for almost complete bioconversion into proteins through fermantation processes. Table 1 summarizes avenues that biotechnology offers for bioconversion of raw materials for animal nutrition.

Table 1. Conversion of biomass for animal nutrition.

PROBLEM	APPROACH
Multiple substrate utilization	Genetic engineering of microbial strains with new metabolic activity
Inaccessibility of substrate	Production and utilization of hydrolytic enzymes
Waste utilization	Increase and upgrade protein content by employing mono- and mixed cultures
Raw material preservation	Convert raw materials into stabilized stocks
Production of low molecular weight feed supplements	New raw materials for microbial production of vitamins and aminoacids

Adoption of particular strategies from those in Table 1 will depend on the raw material to be utilized. This mode of animal feeding can provide for the recycling of soil nutrients and, therefore, offers an avenue for growth of animals compatible with a sustainable environment.

WORKSHOP ON TEACHING OF BIOTECHNOLOGY

Rodolofo Quintero R.

Director, Departomento De Tecnologia De Alimentos
Instituto De Investigaciones Biomedicas
Universidad Nacional De México
Apartado 70228
México, D.F., México

The main topics discussed in the workshop on Teaching of Biotechnology were strategy, international support and specific cases.

STRATEGY

Most developing countries have been and are now concentrating on biotechnology education at the post-graduate level. The main problem has been financing, either for pursuit of local degrees or for sending people abroad. Another big problem has been the stability of employment when people with degrees and training return. In most cases, there is not the infrastructure for their work. Anyway, it seems that most countries in Latin America will push into indigenous Masters and Ph.D. programs.

At the bachelor degree level, an emphasis in biotechnology could be incorporated in the last year of disciplines such as chemistry, biology and chemical engineering. In the long term, such a track will have an important impact, leading to an increase in people with some basic-science knowledge and an understanding of biotechnology. Although some countries have established a bachelor degree in Biotechnology, a new career field is not recommended at this time.

Planning for the development of education and training for biotechnology in Latin American countries was a key issue. Some of the main ideas and aspects were:

1. Planning should look at the short-, medium- and long-term.
2. There must be a clear definition of the specific objectives of the training in biotechnology.
3. A sharp focus is recommended, rather than mere expansion.
4. Each country or region should define its policy taking into account its differences, e.g., size and stage of development, and unique attributes and needs.
5. Trained people should be capable of being productive in their own country, rather than oriented solely to productive efforts in a developed country. Experience is that people who have had working experience in their own countries do a better job when they return after training abroad.
6. Recognize what can be done effectively. Don't do something in biotechnology just for appearance or in search of prestige.

INTERNATIONAL SUPPORT

The American Society for Microbiology has a Latin American Visiting Professorship Program, in which they can provide one to three professors for up to six weeks. The request must originate from a Latin American country and should include courses or conferences. The request should suggest potential visiting professors, themes, etc. Other non-governmental agencies such as AAAS, American Chemical Society and American Institute of Chemical Engineers may help with visiting professors. They also can provide advice of various types.

Many developed countries have bilateral programs for scholarships and visiting professors. In particular, Germany, France and Japan have been active in Latin America.

SPECIFIC CASES

Argentina

A course called Curso Superior de Biologia Molecular y Microbiologia started in May, 1983. It is a twenty-month, masters degree program for 14 students, including up to five from abroad. The program is very interesting in its multi-institutional organization. This program is supported by Comision de Investigaciones Cientificas de la Provincia de Buenos Aires (this institution organizes and directs the program), Subsecretaria de Estado de

Ciencia y Tecnologia y el Consejo Nacional de Investigaciones Cientificas y Tecnicas. Similar courses have been organized, e.g., Food Technology that now is being offered for the second time.

United States

In 1984, MIT will start a new program in biotechnology which will emphasize genetics, physical chemistry, biology, chemistry, chemical engineering and biochemical engineering. It will be a Ph.D. program (3 to 4 years) with an interdisciplinary approach. The purpose is to produce leaders in the field for either universities or industry.

Programs are in planning in Maryland and North Carolina. There are programs of biochemical engineering at MIT, Rutgers, Stanford, California-Berkeley, Maryland, Purdue and Colorado to name a few. There are not now bachelor degree programs in biotechnology in the U.S.A.

Costa Rica

Although there is no biotechnology program, there are postgraduate programs in research that use recombinant DNA, monoclonal antibodies, etc. There is an international course for cloning techniques. There are small efforts in fermentation, e.g., Tecnológico of Cartago.

Mexico

In Mexico, there are twenty-two programs in Biochemical Engineering at the bachelor level and five programs at the masters level, one with ten years experience. There is one program for the Ph.D. in biotechnology. Most of the programs are emphasizing microbiology, enzyme engineering and biochemical engineering.

Industry has started to contact universities for help with solving practical problems and process development. The demand for biotechnologists of high standard is expected to grow.

Brazil

Three years ago, the National Research Council launched a National Program on biotechnology, which involves coordination and integration of efforts of the institutions at each area of the science. Strong funding support for the projects came from the Agency for Technology Financing of Projects (FINEP), based on the established national policy to strengthen the development of research and application of biotechnology. Training by short courses and long-term training or graduate courses are under way at different institutions covering molecular biology, biological nitrogen fixation, monoclonal antibodies, genetic engineeering, plant cell and tissue culture, yeast genetics, plasmid technology, etc.

Collaboration among universities and engineering firms must be encouraged. Training of technical people at all levels is important.

WORKSHOP ON RESEARCH PRIORITIES

Pedro Leon Azofeifa

Centro de Biologia Molecular y Celular
Universidad de Costa Rica
San José, Costa Rica

Before definitive research priorities can be established, it is important to inventory both human and material resources locally and regionally. An inventory should be prepared by each participating nation, pointing out the local availability of "waste" by-products that could be utilized to grow bacteria, fungi or other types of cells. Examples are coffee wastes, bagasse, sawdust and other lignocellulosic compounds. Local conditions that make biotechnological contributions feasible and opportunities to substitute imports should be included. Processes that are not profitable in one place may be profitable somewhere else. Human expertise should be multidisciplinary since several different professions must work together in a coordinated fashion. Strong ties with industry, directly or through technological institutions, universities and governmental agencies (CONICITS, Ministries of Agriculture, etc.) should be encouraged.

Based on the inventories, coordinated actions are needed to determine where biotechnological approaches can be economically and socially relevant at the local level, regionally and in cooperation with developed nations. We recommend that organized groups in the various technological fields carefully study the inventories and meet with public and private industrial concerns to establish feasible ventures.

Biotechnological methodologies should be evaluated in terms of their short-, mid- and long-term possibilities. Strategies that yield short- and mid-term results may be important at this time to create the political support necessary for long-term developments. For example, the application of recombinant technology to plant-genome modification will probably not yield results soon. On the other hand, production of bacterial clones engineered to secrete large quantities of a single type of product, e.g., hormones, vaccines, viral antigens, etc., may be readily applicable with given local conditions and resources.

Schemes that maximize the total potential of modern biotechnologies should be considered. For example, obtaining single-cell protein concomitantly with production of a soluble product of interest will increase the economic feasibility of the project.

WORKSHOP ON TRANSFER OF TECHNOLOGY

Gustavo Viniegra G.

Chief of Microbiology
Division de Ciencias Biologicas y de la Salud
Departamento de Biotecnologia
Universidad Autonoma Metropolitana-Iztapalapa
Avenue Purisima y Michoacan, Col. Vicentina
Iztapalapa, México 13

Science and technology should not be viewed as social objectives in themselves. Rather, they should be a significant part of the overall objectives of a society. In this sense, policy decisions on science and technology should be considered as a result of the dominant forces in a society. This interpretation helps to understand some of the current problems of actual transfer of technology in the various developed and developing nations of our continent. It also underscores the need for scientists and technologists to organize in professional societies to promote their efforts and to gain appreciation and understanding by the rest of society.

Research workers should address specific needs that are fundamental for effective technology transfer:

1. Local research and development capabilities must be strengthened as a prerequisite for appropriate transfer of technology.
2. There is need to integrate basic and applied research and to recognize the strategic role of basic research as the formative nucleus for future technologists involved in appropriate technology transfer.
3. There is need to promote national and international organizations of research workers concerned with actual problems of technology transfer to play an educational role that will orient policy makers, other necessary segments of society, and the research workers themselves, as a necessary step for establishing adequate policies and strategies of technology transfer.
4. Direct contact between research workers and technology users in consulting and practical work must be promoted as a method to enhance the linkage between research and application.
5. Existing non-governmental organizations, such as Interciencia, should be reinforced in performing their roles as a medium for public education on the practical problems and social consequences of the various alternatives of technology transfer.
6. Research workers must be encouraged to participate in the formulation, interpretation and diffusion of the local legal aspects of transfer of technology.

THE REGIONAL SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENT PROGRAM OF THE ORGANIZATION OF AMERICAN STATES

Andres Valeiras

Department of Scientific Affairs
Organization of American States
1889 F Street, N.W.
Washington, D.C. 20006 U.S.A.

The Regional Scientific and Technological Development Program of the Organization of the American States was established in 1968.* It was designed to "advance science and technology to a degree that will contribute substantially to accelerate the economic development and well-being of Latin American people and make it feasible to engage in pure and applied scientific research of the highest possible quality.

"The Program contemplates an objective that has no precedent in Latin America: to intensify, in a coordinated way, the activities in the field of science and technology through the national and regional effort, in accordance with the needs resulting from the insufficient economic and social development in the Latin American countries' goals for expansion and modernization. In Latin America, production processes, through the years, have incorporated technological advances. However, unlike the highly industrialized areas of the world, these new technologies have been incorporated not through these countries' own efforts in research and adaptation, but rather by the simple transfer of such technologies from other countries, without proper consideration being given to the fundamental conditions and requirements of the countries; therefore, without helping to broaden its scientific and technological structure. This procedure has given rise not only to a general technological backwardness in Latin America, but also to serious domestic imbalances: together with the most modern methods of production and consumption, research and education, there are wide areas where methods are primitive and inefficient, and cultural and scientific levels are low.

"The process of advancement is slow and difficult, because of the concurrent action of a number of complex factors, such as 1) the nature of scientific and technological research which requires well-trained personnel and adequate facilities, 2) Latin American present relative backwardness, and 3) the difficulty of setting the objectives of scientific work to meet the overall and sectorial objectives of regional development.

"The Latin American effort for scientific and technological advancement should be based upon educational and cultural objectives and should contribute to the improvement of basic production/consumption conditions by identifying those areas of economic and social development where dissemination of known technology and of original or adaptive research can be most significant. Thus, it cannot be denied that more exact knowledge, as well as an evaluation and protection of Latin America natural resources involve increasing application of present and future scientific and technical resources, that there is backwardness in the quality and quantity of agricultural production, both of food and raw materials for industrial use; and that the manufacturing industry, as compared to that in highly developed countries, is seriously inefficient so that it cannot compete successfully in international markets and/or benefit the domestic consumer. It is evident, also, that Latin America has absorbed only a small part of the scientific advances of recent years and that demographic and social change associated with a growing urbanization pose problems of food, health, housing and human relations that cannot be solved by conventional skills and techniques. Latin America, with vast desert areas and tropical expanses still unexplored, along with temperate zones inadequately exploited, is a fertile field for scientific discovery and development needed to meet the new problems brought about by its growing population and industrial modernization."

In 1974, the Program of Special Projects was established as an important addition to the PRDCT. The principal objective of these projects is the application of educational, scientific and technological infrastructure to the solution of problems deriving from the process of development of the countries, giving attention to the priorities of the overall region or of certain subregions. In this way the need of applying science and technology to development is stressed.

The principal characteristics of the PRDCT follow:

1. It is a complement of the Member States' effort to improve science and technology as well as to apply them to serve the needs of each country. Every two years each Member State proposes a package with the projects to be considered for the next biennium.

**Report of the Group of Experts on Science and Technology to the Inter-American Council on the Regional Scientific and Technological Development Program — Department of Scientific Affairs, General Secretariat of the O.A.S. Washington, D.C., 1967.*

2. The work is based on a multinational structure which uses the horizontal cooperation between the participating countries. This aspect was especially relevant until 1975 and from there on for the special projects.
3. Its action is always directed to help strengthen the institutional infrastructure, providing support for high level training of scientists, technical assistance and laboratory equipment.
4. All the technical activities are handled locally.

During the first 10 years, the PRDCT became one of the most dynamic Inter-American cooperative endeavours and reflected the active role played by the Governments in formulating and designing the programs and in assuming operational responsibilities for conducting and managing the projects.

The General Secretariat's role is basically one of stimulus and technical cooperation, and it serves as a channel of communications through which a network of active relations among the members of the region's scientific and technological community is coordinated. Such relations usually are maintained, even with institutions that have ceased to receive financial support from the Program. With regards to Applied Genetics/Biotechnology, the special projects are well suited because in spite of the basic nature of most of the research which was carried out until now, its applications are always clearly forseen, and such research is generally accompanied by development of technologies needed for concrete applications.

Now, I would like to mention some of the work already done as a base for the work to come. Between 1969 and 1975, the Multinational Project in Genetics worked with three responsible institutions — considered at that time to be among the most developed in the region — in Argentina, Brazil and Chile. These institutions have trained around 60 fellows from 10 different countries at the postgraduate level; most of them worked on thesis for the masters degree and a few of them later received the Ph.D. degree with OAS fellowships. Also, the Program followed through with grants to some of these trainees to start research in their own institutions. Based on the fellows trained, institutions of Bolivia and Perú were incorporated to the project.

After 1975, the work on genetics continued with different institutions in Bolivia, Brazil, Chile and Uruguay. Within the frame of the project, regional activities like a Seminar on Cytogenetics (Montevideo, 1976) and the "Primer Simposio Interamericano sobre Biotecnología de Enzimas" (México, 1981) were held.

The project stimulated and promoted the establishment of national genetic societies and the Latin American Genetics Society, which started holding congresses supported in part by our Program.

In the field, we published 5 monographs — and two others are in preparation — and a document on "Situación de la Genética en la Región" with a directory of 133 institutions in 13 countries and around 1200 geneticists.

From 1975 to 1979, the Special Project on Cytogenetics, working with institutions in Argentina, Chile and Uruguay, was devoted to increasing resources available to man through local development of technology for transplanting mammalian ova in farm animals and through research for biological control of the rodents infected with virus Junín, agent for hemorrhagic fever. As results of the project, in Argentina the techniques developed for embryonic transfer are in use in cattle ranches. The technique is being also used as a tool to improve the quality of camelid herds in a project aimed to rationally exploit these species.

In 1980, we started a Special Project on Enzyme Biotechnology with México. Brazil and Venezuela joined it in 1982 and 1983, respectively. The scope of the work spans from isolation and characterization to production of different enzymes.

The projects on sugarcane, mentioned in round tables at this meeting, and fuel by fermentation both use biotechnologies like some other projects in different sectors of the General Secretary of the OAS.

What are our intentions today? The PRDCT is convening a meeting to design a new special project on Biotechnology. The meeting will be held the second semester of this year and the project should start in 1984, but may possibly be delayed until 1985. All the Member States will be invited to the meeting, but they must submit a concrete proposal in order to attend. The resulting network of the participant institutions will be used for training biologists from all countries.

We hope to help the development of biotechnology in Latin America and the Caribbean through the operation of projects with objectives seeking the solution of very urgent problems in the region. Chagas disease, malaria, and leishmaniasis are endemics affecting millions of people; foot and mouth disease and Newcastle disease produce big losses in cattle and poultry; better and cost-efficient fermentation processes are of paramount importance for improving economic and social conditions of the region; and the improvement of cattle and sheep herds and of different plant species can have a tremendous impact on the economy as a result of a growing capacity to compete in the world markets.

Currently, industries indiscriminately use any available technologies without regard for the environment. The use of biotechnologies in the production system will economically reduce the levels of pollution resulting from such practices.

Through any project of the PRDCT, each country can work to satisfy its own objectives, but the common effort integrated in the multinational network will permit every one to share their capabilities, their capacity to train human resources and the use of the most expensive equipment. The individual experience and expertise will serve to facilitate the work among them and also to reduce the costs of research and development. Furthermore, some of the problems I have mentioned are essentially multinational, and the best solutions should be the ones attained with common work.

A big gap exists already between the developed countries and the Latin American countries, and it will expand in the next years. It is necessary to create the ways to make local research and development a reality in the near future.

ESTABLISHMENT OF A SUB-REGIONAL CENTER FOR RESEARCH AND DEVELOPMENT IN BIOTECHNOLOGY AND GENETICS FOR PHARMACEUTICAL RESEARCH

Francisco Viliesid Alonso

Laboratorios Nacionales de Fomento Industrial
Apartado 41-537
México, D.F., México

In representation of the National Laboratories of Industrial Development of Mexico (Laboratorios Nacionales de Fomento Industrial; LANFI) and of the United Nations Industrial Development Organization (UNIDO), by which I have been asked to prepare this paper, I will briefly, and somewhat chronologically, describe the establishment of a sub-regional center for research and development in biotechnology and genetics for pharmaceutical products. The objective this project pursues is to provide an alternative for the local development of technologies which will permit the production of essential drugs in response to the health needs of the sub-region. Most important among these drugs are the antibiotics.

Upon consideration of the conditions whereby such a development of technology can take place, it was deemed necessary to join the efforts of various countries in a single project. Through this project, the pertinent research and development activities of the participating countries will be coordinated in the pursuit of common goals. One of these goals is reaching a level of technological self-determination for the production of essential drugs.

The project originated at the UNIDO organized seminar of "Microbiological Applications in the Pharmaceutical Industry" of La Habana in 1979. The participants included experts from most countries in Latin America. Because the Latin American region is large and includes diverse economic situations and degrees of development, it was considered necessary to establish at least two sub-regional centers, one to serve Central America, the Caribbean and Mexico, and another to serve South America. The projects for the establishment of these two centers have progressed considerably since 1979, each seeking to respond to sub-regional needs. Soon, both centers will be established in their host countries and will initiate their activities.

The countries participating from the sub-region of Central America, the Caribbean and Mexico so far are: Costa Rica, Cuba, Dominican Republic, Guatemala, Guyana, Honduras, Mexico, Nicaragua and Panama. The international organizations thus far participating are UNIDO and UNDP. These countries and agencies constitute what has been referred to as the Advisory Group.

In August, 1981, this Advisory Group met for the first time in Mexico City. There, it dictated the terms of reference for the UNIDO group of experts in charge of carrying out an Evaluation Mission. The UNIDO experts met with government officials such as industry and health ministers, surveyed teaching and research institutions and visited pertinent local production plants. The mission concluded in January, 1982, at a second Advisory Group meeting. The report submitted by the UNIDO experts was approved. Its recommendations included a list of overall objectives, an organizational structure, guidelines for the programs of activities and training, and location of the Center in Mexico. Mexico, in turn, designated LANFI, a decentralized applied research institution, as the national counterpart and host of the Center.

As from then, and as agreed, LANFI prepared a formal proposal document for the establishment of the center, as well as a draft Constitutive Agreement, both of which were submitted to UNIDO and UNDP. UNDP has expressed its commitment to finance the project, for which UNIDO will act as the international executing agency. Regarding the national counterpart's contributions, Mexico will be the sole financial contributor apart from the UN System. The other participating countries will do so through the collaboration of locally existing research institutions.

Such institutions will form part of the organizational structure of the Center, avoiding costly and unnecessary duplication of efforts.

In the present year, we will see a preparatory assistance phase through which the Center proper will establish design, i.e., objectives, research and training programs, facilities, etc. To this purpose, UNDP has assigned a budget, and UNIDO, in coordination with LANFI, has prepared a program of activities and objectives. To achieve the goals of the preparatory assistance phase, UNIDO is providing experts who, in conjunction with those of the Mexican counterpart, will design the Center. Such a design will necessarily take into account the recommendations of the Advisory Group and of the Evaluation Mission experts. The preparatory assistance phase will conclude at a third Advisory Group meeting by the end of 1983. At this meeting, the design of the Center

will be approved, or accordingly modified, such that the actual establishment and initiation of activities starts in 1984.

In conclusion, UNIDO and UNDP are collaborating with the governments of the sub-region of Central America, the Caribbean and Mexico for the joint establishment of a center for research and development in biotechnology and genetics for pharmaceutical products. The main objectives of this Center are: the local development of technologies for the production of essential drugs, especially those produced through biotechnological processes, in response to the sub-regional health requirements; the adaptation of production to locally available raw materials; and the training of technical personnel for the operation of production plants. The ideas which are fundamental to this project coincide with many of the recommendations expressed at this symposium. Among these are: the need to establish biotechnology centers in Latin America, that these be cooperative and pursue goals common to various countries or sub-regions, the need of improving indigenous capabilities through training, and the production of appropriate technologies through local research and development activities.

CHARACTERISTICS OF A REGIONAL PROJECT TO ESTABLISH A LATIN AMERICAN NETWORK OF BIOTECHNOLOGY CENTERS

Jorge Allende

Departamento de Bioquimica, Universidad de Chile
Santiago, Chile

These ideas were drafted during a mission to the Headquarters of the United Nations Development Program (UNDP, New York) and to UNESCO (Paris). The mission, requested by UNDP and UNESCO, had the objective to discuss the possibilities of organizing a regional project for Latin America in the area of biotechnology.

The proposals contained herein have the "in principle approval" of UNDP and UNESCO, and the partial financing of activities is presently under study by these international agencies.

The proposed project is based on the information and interest gathered by other initiatives such as:

1. UNDP/UNESCO project R.L.A./78/024. Regional Program for Postgraduate Training in Biological Sciences. This 8-year program has operated in 10 countries of South America and has established a network of contacts and information about the biological scientific community of this continent.
2. UNIDO. Project for the establishment of an International Center for Biotechnology and Genetic Engineering. This project, presently under study, has stimulated discussion about the necessity of carrying out some projects in this area.
3. Spain. Project for Scientific and Technological Ibero-American Cooperation being organized as part of the celebration of the 500 Anniversary of the Discoverer of America. This project financed by institutions of the Spanish government has chosen biotechnology as one of the key areas of collaboration.
4. Latin American Countries. Several Latin American countries have set up national projects in the area of biotechnology. Information is available about programs in Argentina, Brazil, Costa Rica, Cuba, Chile and Mexico.

OBJECTIVES OF THE PROJECT

Long-term

To use the techniques of biotechnology to attack problems of health, agricultural production, the use of natural resources and contamination presently facing the Latin American region.

Short-term

1. To strengthen the existing research groups that are using modern techniques of biotechnology to solve problems of special relevance to Latin America.
2. To stimulate the collaboration of the various Latin American groups in research and training.
3. To promote the development of basic sciences related to biotechnology such as microbiology, molecular biology and genetics.
4. To support the creation of new research groups in biotechnology within the region.
5. To inform governmental and industrial institutions about the possibilities and advantages of this new technology.

CRITERIA FOR THE SELECTION OF LABORATORIES AND COUNTRIES INITIALLY PARTICIPATING IN THE PROJECT

In the initial stages of the project, laboratories that could participate would be identified. Each would have several researchers trained in a science related to biotechnology and experience in some of the techniques of genetic engineering, tissue culture of plant or animal cells or the preparation of monoclonal antibodies.

POSSIBLE ACTIVITIES OF THE PROJECT — MINIMAL WORK PLAN

Support of research collaboration

The 15 participating laboratories would receive support consisting of (i) materials and reagents necessary for their research, e.g., US $8000 annually for a total of $120,000, and (ii) travel grants to allow scientific exchange

among participating laboratories, $2,000 for a total of $30,000 annually. This support would be granted for 2 years, depending in the second year on the receipt and approval of the progress report for the first year. After this two year period, a new application period would be opened in which applications from new laboratories would be considered as well.

Information services

The research in biotechnology would be supported by establishing in each participating country a small computer and information service that would have (i) agreements and connection with the large data banks in the developed countries, (ii) computer programs for the rapid analysis of nucleotide sequences and the other research results obtained locally, and (iii) a center of bibliographical information including subscriptions and analysis of biotechnology publications. Also, the centers would provide information retrieval and literature selection facilities. The project would support each center with $10,000 per year.

Gene bank

Within the region, a center would be selected to maintain a collection of microorganisms and animal and plant cells in culture. This laboratory would also maintain a collection of vectors and gene libraries of different microorganisms, animals and plants. This gene bank would provide the strains and gene clones free of cost to the laboratories in the region that would request them for research purposes. This contract would have a cost of $20,000 annually.

Production and distribution of restriction enzymes and polynucleotide synthesis

The project would stimulate various centers in the region to prepare and purify restriction enzymes, synthetic polynucleotides and radioactive precursors that are of common use in gene cloning and genetic engineering. These centers would receive annual contracts that would oblige them to produce a minimum amount of these reagents of adequate purity. The centers would distribute these reagents at minimal or no cost to Latin American laboratories requesting them with the appropriate justification. Estimated cost of each of four contracts is $7,000 per year.

Training postgraduate courses in biotechnology

Annually, three intensive courses (3-4 weeks) would be organized to train young scientists from Latin American countries. These courses would cover specific areas and techniques of biotechnology of particular interest for Latin America. These courses should include at least two visiting professors from international centers of biotechnology and should offer at least eight fellowships to students from Latin American countries. The organizers would receive contracts for $15,000.

Annual biotechnology symposium and workshop

Annually, the project would organize an international symposium or workshop to discuss some aspect of biotechnology of special interest for Latin America and to evaluate the progress of the research in the region. This meeting would attempt to gather researchers from all countries participating in the program to discuss their results and to facilitate the design of collaborative efforts. The organizer would receive a contract to organize a symposium program with the participation of at least 20 researchers from other countries. This contract would have a cost of $25,000.

Long-term fellowships

The project would stimulate the development of biotechnology in countries of the region not originally participating in the project through the granting of six yearly fellowships. The training would be carried out in one of the laboratories participating in the project, would be granted to applicants to undertake formal postgraduate training (M.S. or Ph.D.), and could be renewed for a second or third year. Upon return to their country, these fellows could apply for support for their research projects. The estimated cost of each fellowship is $10,000.

Administration and evaluation

The policy of the project and the annual work plan would be defined by a Regional Executive Council (REC). The REC would be constituted by delegates of the participating governments, by a scientist from each country selected among the leaders of participating laboratories, by the project Technical Coordinator, by a representative of the Latin American Network of Biological Sciences, and by representatives from UNDP and the agencies involved in the execution of the project. The REC meeting place will rotate among the participating countries. The meeting of the REC would be financed with $16,000.

The coordination of the project would be charged to a scientist who has knowledge in the field of biotechnology and who would be contracted as a consultant on a part-time basis. The coordinator would receive one secretary contracted by the project and a small amount of travel and miscellaneous funds for an estimated cost of $40,000 per year.

Annually, the participating countries would be visited by high-level scientific consultants who would evaluate the progress of the research of the laboratories being supported and of the other project activities. The cost would be $10,000.

UNDERUTILIZED FUNDING OPPORTUNITIES FOR RESEARCH IN THE BIOMEDICAL SCIENCES IN LATIN AMERICA

*Cristian Orrego**

Laboratory of Molecular Biology, National Institutes of Health
Building 36, Room 3D02
Bethesda, Maryland 20205 U.S.A.

The participants in the symposium shared the conviction that, for any society, there are profound implications from the field of biotechnology. Many countries are already building their biotechnology base to retain a competitive role in international commerce and as a manufacturing route that promises products and processes that require fewer materials and less non-renewable energy inputs. Shared concern was expressed about the enormous disparities between countries in regard to their capacity to adopt, in an informed manner, this mode of technology. A recent study (1) indicates that, out of 1,372 patents in biotechnology, only a score are held in developing nations, the majority by India. Of about 100,000 microbiologists active today, only a very small fraction are working in the developing countries, primarily in India and Brazil (2).

In the near future and to quote from the program for symposium ". . . this gap may become greater and the only viable alternative could be to purchase the technology or finished products. Unfortunately, very few developing countries will have the resources to acquire either the technology or the products." Indeed, present trends towards patenting of microorganisms and closely guarded research (Dickson, 1980) and the exponentially growing cost of technology transfer in general (3) make it likely that microbial technologies developed abroad will be out of reach for many countries. Faced with this prospect the arrangers of this symposium propose a different strategy, ". . . one in which indigeneous development, however modest initially, stems from local creative effort within a framework of developing-country priorities rather than externally-imposed conditions." I would add that a modest undertaking has to soon be followed by a vast one. Otherwise, many countries will be overwhelmed by the "externally-imposed" technologies that are already resulting from the large commitment to biotechnology by industrialized countries. For example, many of the fuels and chemicals presently derived from petroleum products will likely be produced from biomass by fermentation processes (Gregory, 1982). The added role for agriculture as a materials source for fuel and chemicals poses the undesirable prospect of substituting land presently dedicated to food, for "energy" and "petrochemical farming." As most countries are already experiencing problems associated with soil erosion and nutrient exhaustion as a result of intensive agricultural practices, attempts to develop biomass energy resources will have to be consistent with sustainable food production and soil conservation. This delicate and crucial "balancing act" can only be done well provided there is a creative scientific-technological community familiar with local conditions and capable of working with other organized groups in society. Otherwise "energy farming" will exacerbate malnutrition, the leading health problem in so many countries.

We are faced with the question of how to mobilize scientists and engineers for the development of biotechnology in Latin America. One of the Working Groups in this symposium considered two strategies, development or transfer. I suggest both should be considered and used judiciously. In fact, most of the infrastructure of science and technology that exists today in many countries in Latin America is the result of "transfer" following training of scientists abroad who have returned to and remained in Latin America. I would like to address here one opportunity that is available today to continue this type of "transfer" and which is not being fully taken advantage of.

The United States agency with the primary responsibility for awarding public funds for biomedical research is the National Institutes of Health (NIH). Most of this research has centered on increasing biological knowledge in order to understand and alleviate disease, and to improve human health in general. NIH also funds research in foreign countries directly through its competitive system of extramural research grants and indirectly through the International Research Fellowship Program of the Fogarty International Center. I present evidence here that suggests that both are not being fully availed of by Latin American scientists.

The competition for grants of the NIH is intense and increasing. The number of scientists who are applying for grants has doubled during the past decade, but in real dollars, the allocation of funds by the U.S. Congress has increased only by 50% (Borek, 1982). To qualify for funding, foreign grant applications must fall not only within the normally established payline for that year (the payline is the lowest evaluation score at which grants are funded) but also must be, at a minimum, better than the median of priority scores for approved applications. In

**This article was written by the author in his private capacity. No official support nor endorsement by the National Institutes of Health is intended nor should be inferred.*

1982, NIH received a total of 16,989 applications, 262 of which came from foreign institutions (Table I). Of the former group, 84.9% were recommended for funding while proposals from outside the U.S. received a 75.2% approval rating (Table I). The number of applications actually funded was of course much less for both groups, but in terms of percentages of applications originally submitted, the two values were within 11% of each other (Table I). This indicates that the quality of foreign grant proposals is similar to those generated within the U.S., particularly if it is considered that the former are funded on a more demanding basis than the latter.

Table 1. NIH research project applications reviewed and funded in fiscal year 1982.

	TOTAL	NUMBER		PERCENTAGE OF	
		U.S.	Foreign	U.S.	Foreign
Applications Received	16,989	16,727	262	98.5%	1.5%
Applications recommended for funding	14,396	14,199	197	84.9%	75.2%
Applications funded	4,946	4,897*	49**	29.3%	18.7%

*Total funds = US $560,548,000 which includes indirect costs plus funding of 80 carryover grants (not listed in Table) from previous years.

**Total funds = US $4,072,000 which includes funding of one carryover grant (not listed in Table) from previous years. Indirect costs are not covered in grants to foreign institutions.

The quality of grants originating from South America is also equivalent and in many instances superior, to those being submitted from other countries, many of them enjoying well developed scientific establishments (Table 2). However, the number of research groups from Latin America submitting proposals to NIH has declined during the last five years (Table 2), suggesting underutilization of resources from NIH that could be successfully obtained for biomedical research in the region.

Table 2. NIH research project applications reviewed and funded from foreign institutions.

			TOTAL GRANTS RECEIVED		TOTAL GRANTS RECOMMENDED FOR FUNDING**		TOTAL GRANTS FUNDED	
Fiscal Year	Total Number of Countries	Number of Latin American Countries*	All Countries	Latin American Countries	All Countries	Latin American Countries	All Countries	Latin American Countries
1978	30	7	226	19	160 (70.8)***	12 (63.2)	55 (24.3)***	3 (15.8)
1979	30	6	271	10	185 (68.3)	7 (70.0)	69 (25.4)	1 (10.0)
1980	27	5	269	11	195 (72.4)	4 (36.4)	66 (24.5)	2 (18.2)
1981	28	3	298	7	222 (74.5)	3 (42.8)	77 (25.8)	2 (28.6)
1982	26	3	262	4	197 (25.2)	3 (75.0)	49 (18.7)	2 (50.0)

*Includes Central and South America.

**Number of grants of those received which were reviewed and approved for funding by the National Advisory Council of the NIH.

***Percent of total grants received at NIH.

The International Research Fellowship Program of the Fogarty International Center of NIH provides for postdoctoral training of foreign scientists in the United States. Latin Americans have participated in the program, accounting for 17% (286/1660) of the awards made between 1958 and 1977. One of the criteria of eligibility for this program is nomination by a national committee from the country of origin. Nine countries from Latin America currently participate in the Fogarty Program. In 1982, only 23 nominations were received from them in circumstances that Latin America could be awarded up to 54 fellowhips each year. One factor that contributes to this is the poor publicity of the Program in the U.S. and Latin America. The Program truly generates scientific collaboration between the parties involved. A survey of more than 100 U.S. scientists that worked with Fogarty fellows indicated that more than 80% remained in touch with fellows, and 20% engaged in collaborative research, as documented by original research publications (4). Furthermore, the North American counterpart ". . . had seen their own careers undergo a redirection as a result of the fellowship contact and felt that the large majority of the Latin-American fellows had been as good or better than other postdoctoral fellows they had trained. In addition, there are substantial numbers of examples of the fellow subsequently helping the preceptor establish other Latin American contacts, and the fellow influencing the direction of the preceptor's research" (4). The "brain drain" from countries in Latin America subsequent to training in the U.S. through the Fogarty Program,

has varied from country to country: none for Brazil, 35% for Chile, 6% for Colombia, 2% for Mexico and 69% for Uruguay (4). Therefore, it is difficult to assess, as far as this Program is concerned, the extent of the "loss" of talent that occurs as a result of training abroad. The general paucity of submission of nominations to the Fogarty Program from Latin America, particularly for some countries (Table 3), suggests that here also a resource now available for the development of biotechnology in Latin America is being underutilized.

Table 3. International research fellowship application (Fogarty Program).

DISPOSITION

	1977			1978			1979			1980			1981			1982		
	Received	Approved	Awarded	Received	Approved	Awarded	Received	Approved	Awarded	Received	Approved	Awarded	Received	Approved	Awarded	Received	Approved	Awarded
TOTAL	128	83	67	166	111	88	141	100	82	140	100	64	130	121	96	127	119	94
AMERICAS	18	7	6	28	19	14	23	14	13	25	21	13	21	21	14	23	22	18
Argentina	5	3	3	4	4	3	7	5	4	6	6	4	2	2	1	5	5	5
Brazil	2	1	—	4	4	3	4	4	4	6	4	3	5	5	3	6	6	5
Canada	—	—	—	4	—	—	1	—	—	—	—	—	4	4	3	2	2	—
Chile	4	—	—	6	5	2	4	3	3	6	5	1	2	2	2	5	4	4
Colombia	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Mexico	5	2	2	5	5	5	3	1	1	3	3	2	4	4	4	2	2	2
Peru	1	1	1	3	—	—	—	—	—	3	2	2	2	2	1	1	1	1
Uruguay	—	—	—	2	1	1	3	1	1	1	1	1	2	2	0	2	2	1
Venezuela	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Might we be also underutilizing other resources that U.S. government agencies as well as U.S. scientific societies make available for development of the biomedical sciences in Latin America? We should learn whether or not we are making full use of, for example, the U.S.-Latin America Cooperative Science Program funded by the National Science Foundation, the Program in Science and Technology Cooperation funded by the Agency for International Development, the Grants for Research in Developing Countries administered by the U.S. National Academy of Sciences, the Latin American Professorship Program of the American Society for Microbiology, the Latin American Scholarship Program of American Universities. Are funding opportunities from other nations other than the United States being missed?

The above leads me to invite Interciencia to consider publishing a newsletter for wide dissemination throughout the Americas which would list available international funding opportunities for biotechnology research/training/exchange in Latin America.

Acknowledgements

I am grateful to Dr. Phyllis B. Eveleth and Ms. Elsie Fulton of the Fogarty International Center for making available the information on the NIH international programs presented in this paper.

REFERENCES

Borek, E., (1982): Congress as Research Director. Trends in Biochemical Sciences 7:313-315.

Dickson, D., (1980): Patenting Living Organisms—How to Beat the Bug-Rustlers. Nature 283:128-129.

Gregory, G., (1982): Biotechnology—Japan's Growth Industry. New Scientist 95:308-310.

NOTES

1.–This figure comes from a study by Marstrand P., (1980): "New Developments in Biotechnology and Their Relevance to Environmental Policy," and is quoted in Chopra, R., and Morehouse, W., (1981): "Frontier Technologies, Developing Countries, and the United Nations System after Vienna." Science and Technology, Working Paper Series No. 12, UNITAR.

2.–This figure appears in Gustafson, H., (1980): "Highlights of Technological and Scientific Trends in World Development," and is quoted in Chopra R., and Morehouse, W., (1981): "Frontier Technologies, Developing Countries, and the United Nations System after Vienna." Science and Technology, Working Papers Series No. 12, UNITAR.

3.–See Contreras, C., Edquist, C., Eze, O. C., Morehouse, W., Rangarao, B.V., and Wijeratne, M., (1978): "Technology Transformation of Developing Countries." Discussion Paper 115. Research Policy Program, University of Lund, Sweden.

4.–Brazil: of 33 former fellows all were in full time research and academic activities or working in industry; Chile: of 57 former fellows (5 more could not be located and one more had retired by 1978) all were working in academia, with 20 of them working abroad; Colombia: of 17 former fellows (one more could not be located and one more had died) 11 were engaged in research in the country, four lived in the country but were not active in research and one had moved elsewhere; Mexico: of 40 former fellows (six more could not be located) 39 were engaged in science in the country and one worked for 11 years there before moving abroad; Uruguay: of 26 former fellows only 8 remained in the country involved in research and/or teaching. See, "A Study of Latin-American Countries' Participation in the International Research Fellowship Program of the John E. Fogarty Center for Advanced Study in the Health Sciences, U.S. National Institutes of Health." Survey conducted under Contract No. 263-78-C-0064 of the U.S. Department of Health, Education and Welfare, (December, 1978.)

BIOTECHNOLOGY IN THE AMERICAS: PROSPECTS FOR DEVELOPING COUNTRIES

SUMMARY AND RECOMMENDATIONS

William D. Sawyer

Dean, School of Medicine
Wright State University
Dayton, Ohio 45435 U.S.A.

Initial sessions focused on microbial conversion of biomass to useful products. Important opportunities include: new or improved use of locally plentiful raw materials, improved substrate utilization, use of alternate substrates, the production of by-products of increased value and minimizing or eliminating useless waste products. Many tools are available, for example, microbial genetics, genetic engineering, microbial physiology and fermentation technology.

The key to development in biomass conversion is the identification of limiting steps, whether chemical, cellular or economic. Thereafter, efforts can be specifically directed either to altering favorably or to eliminating the limiting factor. Although genetic engineering has been the key to the excitement about biotechnology, recombinanat DNA procedures are only one of the available technologies and may be the least cost-effective in many situations. It must be emphasized that genetic systems are lacking for many of the industrial microorganisms of current importance. Substantial basic research is needed for the full exploitation of these organisms.

Many believe that agriculture is the field in which biotechnology and genetic engineering will ultimately have the greatest impact. For the present, however, conventional plant breeding and selection techniques coupled with plant cell culture and procedures for cell fusion *in vitro* are the mainstays. Again, much basic research into genetic systems and the physiology of plant cells will be required before genetic engineering can achieve its full potential.

Improved animal health and productivity are other important targets for biotechnology. Some examples are better utilization of food stuffs, conversion of presently non-utilizable materials to food stuffs and techniques for *in vitro* fertilization and embryo transplant. Further, the prospects of more and better vaccines via biotechnology offer improved control of diseases of economically useful animals and, thereby, enhanced production.

In addition to their impact on the economy of the developing countries, developments in biotechnology will contribute directly to improved human health. Substantial advances have already been achieved in the production of drugs, hormones and antibiotics. Many others are forthcoming. In addition, the procedures that make proteins and other macromolecules literally bulk chemicals offer unprecedented technical opportunities for the development of more and better vaccines to prevent infectious diseases that sap the physical and intellectual vigor, especially in the developing countries.

A series of discussions in small groups was a principal process of the symposium. These discussions were intended to promote interactions among the scientists, to enhance knowledge of activities in biotechnology in the various countries, to identify problems and to develop strategies for the application of biotechnology to the problems of the developing countries in the Americas. A summary of those discussions follows.

Significant biotechnological activities are occurring at the industrial level in many of the countries. Operating plants in the developing countries currently produce, for example, ethanol as fuel and as a chemical resource; food and feed additives such as lactic acid, single cell protein, citric acid, monosodium glutamate, and lysine; and pharmaceuticals such as penicillin. Future development depends on proper planning and optimal utilization of local factors and resources. Local resources, opportunities, personnel and markets must be identified and exploited. Judicious selection is required to determine those biotechnological processes that will provide net positive socio-economic returns from investments. Common constraints to most developing countries in the region include limited capital and hard currency and a shortage of skilled labor. In addition, current biotechnology industries are based mainly on traditional technology such as alcoholic fermentation. The realization of the potential of modern biotechnology requires proper managment of the transition from the traditional to the new biotechnology. The economic hazard and human dislocation attendant upon modernizing or revamping the traditional industries must be considered in planning for the introduction of new biotechnology. Further, biotechnology research must ultimately provide benefit to the consumer and to society at large. Linkages between private

industry, non-governmental institutions, and government must be effected. Barriers to effective international cooperation must be resolved by the countries involved.

There was consensus that emphasis should be given to support of national research groups and centers instead of a single large regional center. Networking was stressed. The Latin American and Caribbean countries should, however, also participate in the proposed UNIDO International Center for Biotechnology and Genetic Engineering. Governments should be encouraged to develop policies to stimulate the application of new knowledge provided by basic research. Among suggested processes were: loans for research and development by industries, tax incentives for industrial grants for research and development, and coordinated efforts by scientists to communicate to governments the benefits of investment in biotechnology.

Outstanding achievements are being made in agricultural technology. Vegetative propagation techniques are applied to tropical species such as oil palm, banana, orchid, and ornamentals. Successful application of *in vitro* techniques has eliminated viruses from plant stocks. The technique of plant cell-suspension culture is being explored as a means to produce useful products. The Latin American-Caribbean region as a whole is a net importer of nitrogen fertilizers. More effort should be devoted to better utilization of legumes to promote nitrogen fixation. Remarkable progress in the growth and selection of the "peach palm" was reported in Costa Rica; market development will be important for the future use of this local tree.

In the discussion of human and animal health, it quickly became clear that new, imaginative schemes will be required to develop vaccines of immediate importance mainly in the developing countries. Presently, there is little indication that the established private-sector pharmaceutical industry will independently lead the production of vaccines against many of the important tropical diseases. Because of the difficulty of agreeing on overall priorities, individual nations or groups of nations may need to develop their own resources for the production of vaccines and other pharmaceuticals for human health.

A trio of workshops focused on broad, generic issues; teaching of biotechnology, research priorities and transfer of technology.

Biotechnology brings a new discipline — an interdisciplinary one that is still defining itself, a new name and problems of coordination to the education community. Most developing countries seem to be concentrating on the postgraduate level for teaching biotechnology. A major problem is the opportunity for stable employment when people with advanced degrees seek work in the absence of an appropriate infrastructure in their homeland. For the bachelor degree level, biotechnology should be introduced as a track or option in the last year of the traditional disciplines, rather than as a completely new course of study. Various kinds of international support and specific courses for training in biotechnology were reviewed. The course in molecular biology and microbiology begun in Argentina this year is interesting in that it is focused in multiple institutions. The students travel between them to acquire those aspects of the course in which a given institution is particularly strong. Mexico has twenty-two programs in biochemical engineering at the bachelor level, five programs at the masters level and one program at the Ph.D. level. Most of these programs emphasize microbiology, enzyme engineering or biochemical engineering. Industry has commenced interaction with the Mexican universities in solving practical problems and for process development. Brazil seeks to encourage collaboration among universities and firms in training technical people. There was consensus that research and development institutes ought to concentrate on what they can do well and not try to be all things to all people.

With regard to transfer of technology, science and technology should be considered not as a social objective *per se*, but as a significant part of the overall objectives of a given society. Local research and development capabilities must be strengthened as a prerequisite for appropriate transfer of technology. Basic and applied research must be integrated, and the strategic role of basic research must be recognized as forming the nucleus for future technology. Contact between research workers and technology users should be promoted.

The establishment of priorities for research and development in biotechnology was agreed to be an essential and first task. So many opportunities are available that it will be difficult to determine which ones to focus upon. Inventories must be taken both of resources and of market potential.

Recurring themes were the value of personal contacts and the need to extend the knowledge of biotechnology's role beyond the members of the conference to yet more scientists, to industrial leaders and to appropriate government officials. Governments must be persuaded to an on-going commitment to science and technology as ways to solve important problems. There was consensus that non-governmental organizations such as scientific and engineering societies could be major contributors because of their ability to provide expertise, to evaluate plans and programs, to effect international communication and to develop multinational inventories of resources. Interciencia is a hemispheric, major, non-governmental organization that could be particularly valuable. Intensive efforts should be devoted to expanding the membership of Interciencia, including efforts to include Caribbean nations, and to expanding its programmatic activities. Among suggested activities for Interciencia are an inventory of the people of biotechnology in the Latin American and Caribbean countries; the development of a publication or other mechanism for rapid exchange of information in biotechnology; leadership in developing con-

sensus panels to help to establish national and regional priorities; coordination of training programs in biotechnology; and perhaps, the initiation of a biotechnology network for the developing American nations. Other organizations such as the Organization of American States and the UN agencies, existing networks, and regional centers should be encouraged to participate. Interciencia was urged to begin now by sponsoring a "continuing committee" to promote recommendations of the symposium and to initiate planning for follow-up conferences. In this latter regard, the next conference ought to include officials both from the private sector within the Latin American-Caribbean countries and from government. It was further suggested that experts in the economic aspects of industrial development and in market development be included in future conferences.

The participants agreed that the conference had been of substantial benefit in initiating communication and in setting the scene for the development of collaborative programs. The sponsors and participating agencies are to be commended for supporting this effort.